STIC-ILL

From: Davis, Minh-Tam
Sent: Monday, August 29, 2005 2:41 PM
To: STIC-ILL
Subject: REPRINT REQUEST FOR 10/069973

Bio Tech MAIN
Vol NO X NOS
Dupl Request

8/29

1) INVOLVEMENT OF CYCLOPHILIN - D **IN THE ACTIVATION OF A MITOCHONDRIAL PORE BY CA2+ AND OXIDANT STRESS** (Abstract Available)
Author(s): TANVEER A; VIRJI S; ANDREEVA L; TOTTY NF; HSUAN JJ; WARD JM; CROMPTON M
Corporate Source: UNIV LONDON UNIV COLL,DEPT BIOCHEM & MOLEC BIOL,GOWER ST/LONDON WC1E 6BT//ENGLAND/; UNIV LONDON UNIV COLL,DEPT BIOCHEM & MOLEC BIOL/LONDON WC1E 6BT//ENGLAND/; LUDWIG INST CANC RES/LONDON W1P 8BT//ENGLAND/
Journal: EUROPEAN JOURNAL OF BIOCHEMISTRY, **1996** , V238, N1 (MAY), P166-172
ISSN: 0014-2956

COMPLETED

2) **The mitochondrial permeability transition: its molecular mechanism and role in reperfusion injury.**
Halestrap A P
Department of Biochemistry, University of Bristol, U.K.
Biochemical Society symposium (ENGLAND) **1999** , 66 p181-203, ISSN 0067-8694 Journal Code: 7506896
Publishing Model Print
Document type: Journal Article; Review

3) **mport and processing of heart mitochondrial** cyclophilin D .
Johnson N; Khan A; Virji S; Ward J M; Crompton M
Department of Biochemistry and Molecular Biology, University College London, London, UK.
European journal of biochemistry / FEBS (GERMANY) Jul **1999** , 263 (2) p353-9, ISSN 0014-2956 Journal Code: 0107600

4) **The mitochondrial permeability transition pore and its role in cell death.**
Crompton M
Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, U.K. m.crompton@biochemistry.ucl.ac.uk
Biochemical journal (ENGLAND) Jul 15 **1999** , 341 (Pt 2) p233-49 ISSN 0264-6021 Journal Code: 2984726R
Publishing Model Print

5) **The permeability transition pore complex: a target for apoptosis regulation by caspases and bcl-2-related proteins.**
Marzo I; Brenner C; Zamzami N; Susin S A; Beutner G; Brdiczka D; Remy R; Xie Z H; Reed J C; Kroemer G
Centre National de la Recherche Scientifique, Unite Propre de Recherche 420, F-94801 Villejuif, France.
Journal of experimental medicine (UNITED STATES) Apr 20 **1998** , 187 (8) p1261-71, ISSN 0022-1007 Journal Code: 2985109R

6) Cyclophilin - D **binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore.**
Crompton M; Virji S; Ward J M
Department of Biochemistry and Molecular Biology, University College London, UK. m.crompton@bsm.biochemistry.ucl.ac.uk
European journal of biochemistry / FEBS (GERMANY) Dec 1 **1998** , 258 (2) p729-35, ISSN 0014-2956 Journal Code: 0107600
Publishing Model Print

BEST AVAILABLE COPY

Biochem. Soc. Symp. 66, 181–203
(Printed in Great Britain)

16

The mitochondrial permeability transition: its molecular mechanism and role in reperfusion injury

Andrew P. Halestrap

Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.

Abstract

The mitochondrial permeability transition (mPT) involves the opening of a non-specific pore in the inner membrane of mitochondria, converting them from organelles whose production of ATP sustains the cell, to instruments of death. Here, I first summarize the evidence in favour of our model for the molecular mechanism of the mPT. It is proposed that the adenine nucleotide translocase (ANT) is converted into a non-specific pore through a calcium-mediated conformational change. This requires the binding of a unique cyclophilin (cyclophilin-D, CyP-D) to the ANT, except when matrix [Ca^{2+}] is very high. Binding of CyP-D is increased in response to oxidative stress and some thiol reagents which sensitize the mPT to [Ca^{2+}]. Matrix adenine nucleotides decrease the sensitivity of the mPT to [Ca^{2+}] by binding to the ANT. This is antagonized by carboxyatractyloside (an inhibitor of the ANT) and by modification of specific thiol groups on the ANT by oxidative stress or thiol reagents; such treatments thus enhance the mPT. In contrast, decreasing intracellular pH below 7.0 greatly desensitizes the mPT to [Ca^{2+}]. Conditions which sensitize the mPT towards [Ca^{2+}] are found in hearts reperfused after a period of ischaemia, a process that may irreversibly damage the heart (reperfusion injury). We have demonstrated directly that mPT pores open during reperfusion (but not ischaemia) using a technique that involves entrapment of [^{3}H]deoxyglucose in mitochondria that have undergone the mPT. The mPT may subsequently reverse in hearts that recover from ischaemia/reperfusion, the extent of resealing correlating with recovery of heart function. A variety of agents that antagonize the mPT protect the heart from reperfusion injury, including cyclosporin A, pyruvate and propofol. Mitochondria that undergo the mPT and then reseal may cause cytochrome *c* release and thus initiate apoptosis in cells subjected to stresses less severe than those causing necrosis. An example is the apoptotic cell death in the hippocampus that occurs several days

after insulin-induced hypoglycaemia, and can be prevented by prior treatment with cyclosporin A.

Introduction

Essential for normal mitochondrial function is that the inner membrane remains impermeable to all but a few selected metabolites and ions. If this permeability barrier were to break down within the cell, ATP concentrations could not be maintained, even by glycolysis, since the proton-translocating ATPase of the uncoupled mitochondria would actively hydrolyse rather than synthesize ATP. A cell compromised in this way would be destined to die, since ATP is required to maintain its functional integrity and ionic homoeostasis. Eventually, the permeability barrier of the plasma membrane would be breached through phospholipase A_2 action, and total disruption of cellular metabolite and ion concentrations would make cell death inevitable. Such a non-specific increase in the permeability of the inner mitochondrial membrane (IMM) can occur through a process known as the mitochondrial permeability transition (mPT). This process occurs when the mitochondrial matrix [Ca^{2+}] is greatly increased, especially when this is accompanied by oxidative stress, adenine nucleotide depletion and mitochondrial depolarization. It is caused by the opening of a non-specific pore in the IMM, which transports any molecule of <1500 Da [1–4]. The conditions required to induce the mPT are similar to those experienced by tissues such as the heart when they are reperfused following an extended (>20 min) period of ischaemia [2,4]. It is well known that such reperfusion exacerbates the damage caused by ischaemia itself. Indeed, this reperfusion injury is a major problem associated with open heart surgery where the heart must be stopped during the surgical procedure and then restarted again. In this article I will give a brief review of what is known about the molecular mechanism of the mPT and its regulation. I will then summarize the evidence for a critical role of the mPT in reperfusion injury and how our knowledge of the mechanism of the mPT can provide insights into how the heart might be protected from reperfusion injury.

The molecular mechanism of the mPT

Early data showed that elevated matrix [Ca^{2+}] is required to trigger the mPT, but that the sensitivity to [Ca^{2+}] could be greatly increased by a variety of 'inducers' such as oxidative stress, phosphate, the inhibitor of the adenine nucleotide translocase (ANT) carboxyatractyloside (CAT) and depletion of matrix adenine nucleotides. Sensitivity to [Ca^{2+}] is reduced by other factors such as ADP, low pH and bongkrekic acid (BKA) [4–7]. All solutes <1500 Da enter the mitochondria following the mPT and the process is reversed immediately by chelation of Ca^{2+} with EGTA [6–9]. Although these data clearly imply the presence of a Ca^{2+}-dependent, non-selective channel in the IMM, for many years it was argued that a phospholipase A_2-mediated increase in the permeability of the inner membrane phospholipid bilayer was responsible [10]. The

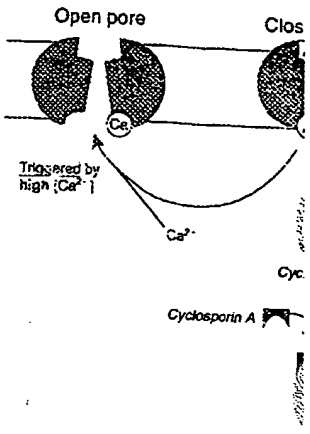

Figure 1 Proposed model for the the absence of CyP-D binding to the [Ca^{2+}], but binding of CyP-D enables occur at lower [Ca^{2+}]. CyP binding reagents, chaotropic agents and increa the mPT to [Ca^{2+}]. In contrast, adenir face of the ANT, which is enhanced by desensitizes the mPT to [Ca^{2+}]. Adeni 'c' conformation of ANT, thiol reagent the mPT to occur at higher $\Delta\Psi$ and lo probably inhibit pore opening by comp site.

one observation more than any other t onstration by Crompton and colleagu the mPT can be specifically and totally tions of cyclosporin A (CsA) without [11–13]. CsA is a drug with powerful in ated through an inhibition of calc phosphatase, by a complex formed b (CyP), CyP-A [14]. This suggested to u also involve a member of the CyP fam studies in this and other laboratories ha led us to propose a mechanism for the involves the formation of a non-specific formational change of the ANT. It is su Ca^{2+} and facilitated by the binding of tein with peptidylprolyl *cis–trans* isome model and the mechanisms by which fa act are presented later and are summariz

nd can be prevented by prior treatment

ial function is that the inner membrane
elected metabolites and ions. If this per-
n within the cell, ATP concentrations
ycolysis, since the proton-translocating
ia would actively hydrolyse rather than
in this way would be destined to die,
unctional integrity and ionic homoeosta-
ier of the plasma membrane would be
action, and total disruption of cellular
ould make cell death inevitable. Such a
ity of the inner mitochondrial membrane
nown as the mitochondrial permeability
when the mitochondrial matrix [Ca^{2+}] is
; is accompanied by oxidative stress, ade-
ondrial depolarization. It is caused by the
IMM, which transports any molecule of
uired to induce the mPT are similar to
ie heart when they are reperfused follow-
schaemia [2,4]. It is well known that such
caused by ischaemia itself. Indeed, this
associated with open heart surgery where
ie surgical procedure and then restarted
review of what is known about the molec-
s regulation. I will then summarize the
mPT in reperfusion injury and how our
: mPT can provide insights into how the
usion injury.

of the mPT

ed matrix [Ca^{2+}] is required to trigger the
$^{2+}$] could be greatly increased by a variety
s, phosphate, the inhibitor of the adenine
oxyatractyloside (CAT) and depletion of
rity to [Ca^{2+}] is reduced by other factors
dic acid (BKA) [4–7]. All solutes <1500 Da
e mPT and the process is reversed immedi-
[A [6–9]. Although these data clearly imply
ion-selective channel in the IMM, for many
lipase A_2-mediated increase in the perme-
pholipid bilayer was responsible [10]. The

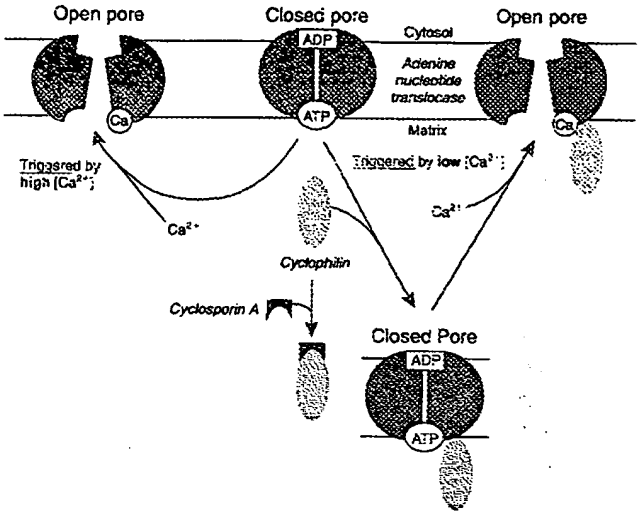

Figure 1 Proposed model for the mechanism of action of the mPT. In the absence of CyP-D binding to the ANT, the mPT can still open at high [Ca^{2+}], but binding of CyP-D enables the required conformational change to occur at lower [Ca^{2+}]. CyP binding is increased by oxidative stress, thiol reagents, chaotropic agents and increased matrix volume, which all sensitize the mPT to [Ca^{2+}]. In contrast, adenine-nucleotide binding to the matrix surface of the ANT, which is enhanced by the 'm' conformation of ANT and $\Delta\Psi$, desensitizes the mPT to [Ca^{2+}]. Adenine-nucleotide binding is inhibited by the 'c' conformation of ANT, thiol reagents and oxidative stress, which thus allow the mPT to occur at higher $\Delta\Psi$ and lower [Ca^{2+}]. [Mg^{2+}] and [H^+] (low pH) probably inhibit pore opening by competing directly with Ca^{2+} for the trigger site.

one observation more than any other that overturned this view was the demonstration by Crompton and colleagues, and subsequently many others, that the mPT can be specifically and totally inhibited by submicromolar concentrations of cyclosporin A (CsA) without any effect on phospholipase A_2 activity [11–13]. CsA is a drug with powerful immunosuppressive activity that is mediated through an inhibition of calcineurin, a calcium-sensitive protein phosphatase, by a complex formed between CsA and cytosolic cyclophilin (CyP), CyP-A [14]. This suggested to us that effects of CsA on the mPT might also involve a member of the CyP family within the matrix [13]. Subsequent studies in this and other laboratories have confirmed this suggestion and have led us to propose a mechanism for the mPT, shown in Figure 1. Our model involves the formation of a non-specific channel occurring as a result of a conformational change of the ANT. It is suggested that this process is triggered by Ca^{2+} and facilitated by the binding of another matrix protein, CyP-D, a protein with peptidylprolyl *cis–trans* isomerase (PPIase) activity. Evidence for this model and the mechanisms by which factors known to regulate the mPT may act are presented later and are summarized in Tables 1 and 2 respectively.

Table 1 Summary of the evidence for a role for the ANT and CyP in the formation of the PT pore

Details are presented in the text. TBH, t-butylhydroperoxide.

CyP involvement

- Mitochondria contain a unique nuclear-encoded CyP, CyP-D, with peptidyl-prolyl *cis–trans* isomerase PPIase activity that is inhibited by CsA
- The K_i for inhibition of this PPIase activity by a range of CsA analogues matches their $K_{0.5}$ values for inhibition of the mPT
- CyP-D binds in a CsA-sensitive manner to the IMM. Binding is increased by oxidative stress, thiol reagents, chaotropic agents and increased matrix volume that also stimulate the mPT

ANT involvement

- The mPT is sensitized to $[Ca^{2+}]$ by CAT, which locks the ANT in the 'c' conformation, and is inhibited by BKA, which locks the ANT in the 'm' conformation
- The mPT is inhibited by micromolar ADP concentrations in the matrix, and this effect is antagonized by CAT. Only nucleotides that bind to the ANT inhibit the mPT
- Oxidative stress (TBH) and thiol reagents (diamide, phenylarsine oxide, eosine maleimide) stimulate the mPT and reduce the effectiveness of ADP as an inhibitor of the mPT by two orders of magnitude or more
- The ANT is known to have thiol groups that are reactive towards oxidative stress and phenylarsine oxide. Eosine maleimide specifically modifies Cys^{159} of the ANT
- The ANT is an electrogenic carrier and is thus a suitable candidate for the membrane potential sensor of the mPT. Opening of the mPT in adenine nucleotide-depleted mitochondria is insensitive to the membrane potential
- The purified and reconstituted ANT can form a non-selective channel at high $[Ca^{2+}]$ or when specific thiol groups are modified. Although similar to the mPT pore, it is insensitive to CsA

An ANT–CyP-D complex

- The purified ANT and that of solubilized IMMs binds tightly and in a CsA-inhibitable manner to immobilized GST–CyP-D
- The purified ANT, reconstituted in the presence of the GST–CyP-D, can form a non-selective channel at low $[Ca^{2+}]$ that is inhibited by CsA

Techniques for measuring the mPT in isolated mitochondria

All investigations on the mechanism of the mPT require the use of a reliable assay method and several are in common use. The most direct is the sucrose-entrapment technique introduced by Crompton and co-workers in which permeation of [^{14}C]sucrose (or other solutes) into the matrix is determined [8,9,15,16]. Using rapid reaction techniques this method has been used to characterize the kinetics and specificity of the pore, but is relatively cumbersome and does not allow continuous monitoring of the process. The most commonly used continuous assay utilizes the decrease in light scattering that occurs as mitochondria swell during the mPT [5–7,13]. Swelling takes place as small-molecular-mass solutes equilibrate through the non-specific pore while higher-molecular-mass solutes continue to exert an osmotic pressure which

Table 2 Proposed sites of act

Note that both CyP and ADP bin
sensitivity of the mPT to $[Ca^{2+}]$.
reagents such as phenylglyoxal ha
inhibit the mPT induced by a wide
oxide; TBH, t-butylhydroperoxid

Effect via change in CyP-D binding	Effec in nu
Activatory	
Thiol reagents (e.g. diamide, PheArs)	Thiol (e.g.
Oxidative stress (e.g. TBH)	Oxid (e.g.
Increased matrix volume	'c' c
Chaotropic agents	
Inhibitory	
CsA	Men
	Men
	(e.g
	'm'
	Argi

drives water into the matrix. A m
mPT pore by exposure of mitocho
chondrial shrinkage that occurs up
too large to enter the matrix throu
technique is that it allows the con
modified when effectors of the m
studied. Another continuous ass
decrease in mitochondrial transme
mulated matrix Ca^{2+} caused by the
and Ca^{2+} that occurs during the m
sensitive electrodes and dyes, bu
uncoupling of the mitochondria b
Discrimination is achieved throug
problems. There is now evidence t
that transport H^+ and Ca^{2+} but no
that the mPT becomes CsA-insensi

A major problem of many pu
anism of the mPT is that the m
between factors directly affecting t
rectly through changes in other
membrane potential. In our own la
the number of variables that may c
have usually used de-energized mit

ole for the ANT and CyP in

hydroperoxide.

ded CyP, CyP-D, with peptidyl-
inhibited by CsA
a range of CsA analogues
PT
e IMM. Binding is increased by
nts and increased matrix volume

ich locks the ANT in the 'c' con-
the ANT in the 'm'

ncentrations in the matrix, and
ides that bind to the ANT inhibit

amide, phenylarsine oxide,
ce the effectiveness of ADP as an
de or more
are reactive towards oxidative
e specifically modifies Cys^{159} of

is a suitable candidate for the
ng of the mPT in adenine
to the membrane potential
n a non-selective channel at high
ied. Although similar to the mPT

ds binds tightly and in a
yP-D
nce of the GST–CyP-D, can
t is inhibited by CsA

lated mitochondria

the mPT require the use of a reli-
non use. The most direct is the
y Crompton and co-workers in
solutes) into the matrix is deter-
niques this method has been used
he pore, but is relatively cumber-
toring of the process. The most
e decrease in light scattering that
[5–7,13]. Swelling takes place as
ough the non-specific pore while
exert an osmotic pressure which

Table 2 Proposed sites of action of known modulators of the mPT

Note that both CyP and ADP binding exert their effects through changes in the sensitivity of the mPT to $[Ca^{2+}]$. The locus of action of arginine-specific reagents such as phenylglyoxal has not been defined but these agents appear to inhibit the mPT induced by a wide range of effectors. PheArs, phenylarsine oxide; TBH, t-butylhydroperoxide.

Effect via change in CyP-D binding	Effect via change in nucleotide binding	Direct effect on Ca^{2+} binding
Activatory		
Thiol reagents (e.g. diamide, PheArs)	Thiol reagents (e.g. diamide, PheArs)	High pH
Oxidative stress (e.g. TBH)	Oxidative stress (e.g. TBH)	
Increased matrix volume	'c' conformation of ANT	
Chaotropic agents		
Inhibitory		
CsA	Membrane potential	Low pH
	Membrane surface charge (e.g. trifluoperazine)	Mg^{2+}
	'm' conformation of ANT	Arginine reagents
	Arginine reagents	

drives water into the matrix. A modification of this assay is to fully open the mPT pore by exposure of mitochondria to high $[Ca^{2+}]$ and then measure mitochondrial shrinkage that occurs upon addition of poly(ethylene glycol) that is too large to enter the matrix through the pore [7,17–19]. The advantage of this technique is that it allows the composition of the mitochondrial matrix to be modified when effectors of the mPT working on the matrix surface are to be studied. Another continuous assay that has been employed follows the decrease in mitochondrial transmembrane potential ($\Delta\Psi_m$) or release of accumulated matrix Ca^{2+} caused by the massive increase in permeability to protons and Ca^{2+} that occurs during the mPT [3,10]. This can be determined using ion-sensitive electrodes and dyes, but suffers from the disadvantage that any uncoupling of the mitochondria by other causes will produce a similar result. Discrimination is achieved through the use of CsA, but this is not without its problems. There is now evidence that there may be substates of the mPT pore that transport H^+ and Ca^{2+} but not larger solutes [20,21], and it is also known that the mPT becomes CsA-insensitive when $[Ca^{2+}]$ is high [18,19,22–24].

A major problem of many published studies on the regulation and mechanism of the mPT is that the methods used do not allow discrimination between factors directly affecting the mPT mechanism and those working indirectly through changes in other parameters such as matrix $[Ca^{2+}]$ and membrane potential. In our own laboratory we have endeavoured to simplify the number of variables that may contribute to the regulation of the mPT and have usually used de-energized mitochondria, more recently in the presence of

the calcium ionophore A23187 [13,17–19,24]. This ensures that $\Delta\Psi_m$ and matrix $[Ca^{2+}]$ are held constant, and thus factors influencing the mPT cannot be doing so indirectly through these parameters. We have also used a very sensitive continuous assay of light scattering to determine mitochondrial swelling and shrinkage, which has allowed the determination of mPT kinetics [13,19,24].

The role of mitochondrial CyP in the mPT

We and others have demonstrated that the mitochondrial matrix contains a PPIase that is inhibited by CsA and its analogues with $K_{0.5}$ values very similar to those for inhibition of the mPT [13,24–27]. Furthermore, the number of binding sites required for 100% inhibition of the mPT corresponds to the concentration of PPIase within the matrix (about 50 pmol per mg of protein) [13,25]. Purification and N-terminal sequencing of this PPIase confirmed that it was a member of the CyP family, most probably identical to the product of the human CyP-3 gene [28,29]. This nuclear-encoded protein is now more usually termed CyP-D and has a mitochondrial targeting presequence that is cleaved after translocation of the protein into the matrix. We have cloned and sequenced the cDNA for rat mitochondrial CyP (accession number U68544) and, with the exception of the extreme N-terminal residue, the sequence corresponds exactly to the N-terminus sequence of the purified protein [27,28]. This confirms that the matrix PPIase in rat mitochondria is indeed the equivalent of human CyP-3. Removal of the mitochondrial targeting presequence from the CyP-D takes place in the matrix and may occur at one of two points leading to mature proteins of about 17.6 kDa (minor product) and 18.6 kDa (major product) [27,28]. Crompton and colleagues have also purified and sequenced a mitochondrial CyP that is associated with the IMM and which proved to be CyP-D [30,31]. We have used Northern blotting to show that mRNA for CyP-D in rat muscle, heart, liver, kidney and brain is of identical size (1.5 kb), making it unlikely that there are differently spliced tissue-specific isoforms [28].

A wide range of inducers can sensitize the mPT to $[Ca^{2+}]$, and our model suggests that one way this might be achieved is by enhancing CyP-D binding to the membrane component(s) of the mPT pore. To investigate this possibility we developed a method to measure the amount of CyP-D bound to IMMs. This involved the rapid isolation of IMMs following treatment of mitochondria with inducers that enhance the sensitivity of the mPT to $[Ca^{2+}]$, followed by SDS/PAGE and Western blotting with anti-CyP-D antibodies [17,18]. Oxidative stress induced with t-butylhydroperoxide (TBH), glutathione depletion induced by diamide treatment or modification of vicinal thiols by phenylarsine oxide (PheAs) were all shown to increase CyP binding to the IMM concomitant with their ability to increase the sensitivity of pore opening to $[Ca^{2+}]$ [17–19]. Mild chaotropic agents such as KSCN and increases in matrix volume were also able to increase CyP binding in parallel with their ability to sensitize the mPT to $[Ca^{2+}]$ [18]. In all cases, binding of CyP was almost totally prevented by CsA. In contrast, we found that several other modulators of the mPT, such as matrix $[Ca^{2+}]$, [ADP], pH or membrane potential

were without effect on CyP bindi
ferent techniques, have obtained c

Although the evidence for a
is strong, there is a body of data
essential for the opening of the r
CyP-D may be to facilitate a confc
would only occur at very high [C
of pore opening by CsA is over
[18,19,22–24]. Yet under the sam
totally the binding of CyP-D to
ANT is capable of behaving as a r
as will be described later.

The role of the ANT in the mPT

The ANT was first implicate
reagent, such as CAT, that stabiliz
ANT, stimulated the mPT, wher
conformation of the ANT, inhibit
ADP is an important modulator
sensitivity of the calcium trigger si
ing sites with K_i values of about
CAT [19,22,34]. We have tested th
the mPT, and found that only AT
being, respectively, 500 and 20 tir
their affinity for the matrix-bindir
binding is antagonized by oxidativ
by thiol reagents such as PheArs a
powerful activators of the mPT
reagents tested, raising the $K_{0.5}$ fo
this is accompanied by covalent r
eosine maleimide is known to atta
the translocase by preventing ADP

Bernardi and colleagues have
the mPT by the $\Delta\Psi_m$, activation
[37–40]. We have demonstrated
nucleotides by pyrophosphate trea
sitive to $[Ca^{2+}]$, but it is also no
regulation of the mPT by $\Delta\Psi_m$ is d
nucleotides and may involve the
effect on adenine nucleotide bindin
porting ATP^{4-} in exchange for A
potential-driven conformational cl
nucleotides on either side of the m
enhance ATP binding to the ANT
shifts the voltage dependence of th
negative potentials. Two distinct
effect, one sensitive to oxidation

,24]. This ensures that $\Delta\Psi_m$ and
actors influencing the mPT cannot
:ters. We have also used a very sen-
) determine mitochondrial swelling
determination of mPT kinetics

'T
t the mitochondrial matrix contains
logues with $K_{0.5}$ values very similar
-27]. Furthermore, the number of
)f the mPT corresponds to the con-
bout 50 pmol per mg of protein)
ıcing of this PPIase confirmed that
robably identical to the product of
-encoded protein is now more usu-
lrial targeting presequence that is
to the matrix. We have cloned and
l CyP (accession number U68544)
rminal residue, the sequence corre-
of the purified protein [27,28]. This
:hondria is indeed the equivalent of
ial targeting presequence from the
:cur at one of two points leading to
roduct) and 18.6 kDa (major prod-
ve also purified and sequenced a
the IMM and which proved to be
tting to show that mRNA for CyP-
brain is of identical size (1.5 kb),
ly spliced tissue-specific isoforms

the mPT to $[Ca^{2+}]$, and our model
ed is by enhancing CyP-D binding
pore. To investigate this possibility
ıount of CyP-D bound to IMMs.
)llowing treatment of mitochondria
)f the mPT to $[Ca^{2+}]$, followed by
anti-CyP-D antibodies [17,18].
peroxide (TBH), glutathione deple-
nodification of vicinal thiols by
vn to increase CyP binding to the
ease the sensitivity of pore opening
; such as KSCN and increases in
CyP binding in parallel with their
. In all cases, binding of CyP was
t, we found that several other mod-
[ADP], pH or membrane potential

were without effect on CyP binding [18,19], although other workers, using different techniques, have obtained conflicting results [30–32].

Although the evidence for a role of CyP-D in the mPT presented earlier is strong, there is a body of data that imply that CyP-D binding may not be essential for the opening of the mPT pore. Rather, it seems that the role of CyP-D may be to facilitate a conformational change of the ANT that otherwise would only occur at very high $[Ca^{2+}]$. Thus, at high matrix $[Ca^{2+}]$, inhibition of pore opening by CsA is overcome in both heart and liver mitochondria [18,19,22–24]. Yet under the same conditions, CsA is able to prevent almost totally the binding of CyP-D to the IMM [17,19]. Furthermore, the purified ANT is capable of behaving as a non-specific pore at high (millimolar) $[Ca^{2+}]$ as will be described later.

The role of the ANT in the mPT

The ANT was first implicated in the mPT when it was observed that any reagent, such as CAT, that stabilized the 'c' (cytoplasmic) conformation of the ANT, stimulated the mPT, whereas BKA, which stabilized the 'm' (matrix) conformation of the ANT, inhibited the mPT [13,19,33]. Furthermore, matrix ADP is an important modulator of pore opening that acts by decreasing the sensitivity of the calcium trigger site to $[Ca^{2+}]$ [6,19]. There are two ADP binding sites with K_i values of about 1 and 25 μM, the former being blocked by CAT [19,22,34]. We have tested the ability of a range of nucleotides to inhibit the mPT, and found that only ATP and deoxy-ADP inhibit, their $K_{0.5}$ values being, respectively, 500 and 20 times greater than ADP. This correlates with their affinity for the matrix-binding site of the ANT [19]. Adenine nucleotide binding is antagonized by oxidative stress induced by TBH or diamide and also by thiol reagents such as PheArs and eosine maleimide, which are known to be powerful activators of the mPT [19]. PheArs has the greatest effect of the reagents tested, raising the $K_{0.5}$ for ADP inhibition of the mPT to >500 μM; this is accompanied by covalent modification of the ANT [18]. Furthermore, eosine maleimide is known to attack Cys^{159} of the ANT and inhibit activity of the translocase by preventing ADP binding to the matrix-binding site [35,36].

Bernardi and colleagues have provided strong evidence for regulation of the mPT by the $\Delta\Psi_m$, activation occurring as $\Delta\Psi_m$ becomes less negative [37–40]. We have demonstrated that in mitochondria depleted of adenine nucleotides by pyrophosphate treatment, not only is the mPT much more sensitive to $[Ca^{2+}]$, but it is also no longer sensitive to $\Delta\Psi_m$ [19,25]. Thus, the regulation of the mPT by $\Delta\Psi_m$ is dependent on the presence of matrix adenine nucleotides and may involve the ANT itself responding to $\Delta\Psi_m$ through an effect on adenine nucleotide binding. The ANT is an electrogenic carrier, transporting ATP^{4-} in exchange for ADP^{3-}. Its mechanism may well involve a potential-driven conformational change that alters the affinity of the adenine nucleotides on either side of the membrane [41–43]. A large negative $\Delta\Psi_m$ will enhance ATP binding to the ANT and thus inhibit the mPT. Oxidative stress shifts the voltage dependence of the mPT, allowing the pore to open at more negative potentials. Two distinct thiol groups have been implicated in this effect, one sensitive to oxidation of glutathione, for example by TBH or

diamide, and the other responding to the redox state of matrix NAD(P) [39,44,45]. The ANT is known to have three cysteine residues that show differential reactivity to various thiol reagents in a conformation-dependent manner [35,36]. These are Cys^{56}, Cys^{159} and Cys^{256} and, as discussed earlier, it seems likely that modification of Cys^{159} is responsible for the ability of oxidative stress and thiol reagents to reduce the inhibitory effects of ADP and membrane potential on the mPT [19]. Modification of Cys^{56} is probably responsible for the effects of oxidative stress and thiol reagents on CyP-D binding to the ANT [19]. Bernardi and colleagues have recently demonstrated that the arginine-specific reagents phenylglyoxal and 2,3-butanedione strongly favour the closed state of the mPT pore, even in the presence of strong inducers such as high $[Ca^{2+}]$ and ADP depletion [46,47]. The location of the arginine groups responsible for this effect has not been established but they are accessible from the matrix surface and the ANT has a large number of arginines on the three matrix-facing loops.

Although a role for the ANT in the mPT is now generally accepted (see [1–3]), there has been debate as to whether this protein may itself form the pore or rather be a regulatory component. In particular, there was no direct evidence for an interaction of CyP-D with the ANT. Our own attempts at chemical cross-linking of the CyP-D to the ANT have so far met with no success. As an alternative strategy we have overexpressed CyP-D as a glutathione S-transferase (GST) fusion protein and used this to investigate what protein(s) of the IMMs bind to it [4,48]. We first demonstrated that the purified GST–CyP-D fusion protein could bind to IMMs in a CsA-sensitive manner and that this binding was enhanced by diamide treatment. We then solubilized the IMMs in Triton X-100 and incubated these with GST–CyP-D immobilized on Sepharose. Bound proteins were washed extensively and then eluted with glutathione and analysed by SDS/PAGE and Western blotting with anti-ANT and porin antibodies. A major protein band of 30 kDa was found to bind in a CsA-sensitive manner and this correlated with the presence of a strong ANT-reactive band on the Western blot. Binding of the ANT was prevented by both CAT and BKA, suggesting that it depended on the ANT undergoing conformational change. No porin binding was observed under the same conditions [4,48].

Other possible components of the mPT pore

The evidence presented earlier confirms an interaction of CyP-D with the ANT, but this does not allow us to conclude that the ANT and CyP-D alone are sufficient to form the mPT pore. It has been reported that just the purified and reconstituted ANT alone can form Ca^{2+}-dependent channels resembling the PT pore [49,50], but the opening of these channels required concentrations of $[Ca^{2+}]$ of more than 0.5 mM and was not CsA-sensitive. However, we have recently reconstituted the purified ANT into proteoliposomes in the presence of GST–CyP-D and measured Ca^{2+}-dependent pore opening by the release of entrapped malate (A. Rück, J. Gillespie, D. Brdiczka and A. Halestrap, unpublished work). Whereas in the absence of GST–CyP-D the purified ANT could be induced to form a pore only at $[Ca^{2+}]>0.25$ mM [50], in its presence only

5–10 μM Ca^{2+} was required [48]. Fu
GST–CyP-D became sensitive to i
absence [48]. Thus it does appear th
pore formation is the presence of A
[13], although this does not exclude
in a regulatory manner. A particul
resolved is where the calcium trigge
binding motifs on either the ANT
opening occurs in the ANT–CyP-I
calcium-binding proteins. We have
molar concentrations or less can in
[13,51] and thus it seems most likely
ated with the matrix loops of th
aspartates and glutamates in the thr
such a way as to produce a calcium-l

Those advocating a central ro
the mPT pore complex includes th
the benzodiazepine receptor (see
reports that these components ma
under some conditions [54,55], our
direct involvement in the pore con
ented evidence that argues against th
First, ligands of the mitochondrial
direct effect on the mPT [19]. Seco
from which the outer membrane ha
ment and exhibits identical properti

The locus of action of different m

In Table 2 I summarize how t
the action of the major modulator
nucleotides to the ANT inhibits pc
the trigger site for $[Ca^{2+}]$. Adenine
conformation of the carrier, memb
attack Cys^{159} of the ANT. These a
the 'm' conformation and increase
binding and inhibit the pore. These
recruitment to the membrane. Chac
sensitize the mPT to $[Ca^{2+}]$ by inc
reagents and oxidative stress also
Cys^{56} of the ANT [18]. This facilita
channel state. Low pH (<7.0) and ir
mPT [7,18,19,56,57] and appear to
binding at the trigger site. Bernardi
that the effect of low pH involves a
modulate CyP binding to the IMM
did not detect such an effect of low
branes [18] or to the purified ANT

redox state of matrix NAD(P)
ysteine residues that show differ-
conformation-dependent manner
and, as discussed earlier, it seems
sible for the ability of oxidative
ory effects of ADP and membrane
Cys^{56} is probably responsible for
ts on CyP-D binding to the ANT
demonstrated that the arginine-
iedione strongly favour the closed
of strong inducers such as high
on of the arginine groups respon-
but they are accessible from the
umber of arginines on the three

PT is now generally accepted (see
s protein may itself form the pore
ular, there was no direct evidence
. Our own attempts at chemical
so far met with no success. As an
CyP-D as a glutathione S-trans-
investigate what protein(s) of the
d that the purified GST–CyP-D
A-sensitive manner and that this
We then solubilized the IMMs in
GST–CyP-D immobilized on
nsively and then eluted with glu-
Western blotting with anti-ANT
of 30 kDa was found to bind in a
h the presence of a strong ANT-
the ANT was prevented by both
on the ANT undergoing confor-
erved under the same conditions

ore

an interaction of CyP-D with the
that the ANT and CyP-D alone
en reported that just the purified
-dependent channels resembling
channels required concentrations
CsA-sensitive. However, we have
proteoliposomes in the presence
nt pore opening by the release of
diczka and A. Halestrap, unpub-
–CyP-D the purified ANT could
.25 mM [50], in its presence only

5–10 μM Ca^{2+} was required [48]. Furthermore, pore opening in the presence of GST–CyP-D became sensitive to inhibition by CsA, which it was not in its absence [48]. Thus it does appear that the minimum requirement for the mPT pore formation is the presence of ANT and CyP-D, as we originally proposed [13], although this does not exclude the possibility of other components acting in a regulatory manner. A particularly interesting question that needs to be resolved is where the calcium trigger site is located. There are no obvious Ca^{2+}-binding motifs on either the ANT or CyP-D, yet calcium-dependent pore opening occurs in the ANT–CyP-D complex without the need for additional calcium-binding proteins. We have demonstrated that matrix calcium at micromolar concentrations or less can induce a conformational change in the ANT [13,51] and thus it seems most likely that there is a calcium-binding site associated with the matrix loops of this protein. There are several conserved aspartates and glutamates in the three matrix loops and these may orientate in such a way as to produce a calcium-binding motif.

Those advocating a central role of the mPT in apoptosis often state that the mPT pore complex includes the outer membrane components, porin and the benzodiazepine receptor (see for example [52,53]). Whereas there are reports that these components may co-purify with the ANT as a complex under some conditions [54,55], our own studies provide no support for their direct involvement in the pore complex [19,48]. Furthermore, we have presented evidence that argues against their playing a role in regulation of the mPT. First, ligands of the mitochondrial benzodiazepine receptor are without any direct effect on the mPT [19]. Second, the mPT can be observed in mitoplasts from which the outer membrane has been largely removed by digitonin treatment and exhibits identical properties to the mPT in normal mitochondria [19].

The locus of action of different modulators of the mPT

In Table 2 I summarize how the ANT may provide a common locus for the action of the major modulators of the mPT. Binding of matrix adenine nucleotides to the ANT inhibits pore formation by decreasing the affinity of the trigger site for $[Ca^{2+}]$. Adenine nucleotide binding is antagonized by the 'c' conformation of the carrier, membrane depolarization and thiol reagents that attack Cys^{159} of the ANT. These all sensitize the mPT to $[Ca^{2+}]$. In contrast, the 'm' conformation and increased membrane potential enhance nucleotide binding and inhibit the pore. These effectors are all without effect on CyP-D recruitment to the membrane. Chaotropic agents and increased matrix volume sensitize the mPT to $[Ca^{2+}]$ by increasing CyP-D binding, an effect that thiol reagents and oxidative stress also induce, perhaps through modification of Cys^{56} of the ANT [18]. This facilitates the transition of the ANT into its open channel state. Low pH (<7.0) and increased $[Mg^{2+}]$ are potent inhibitors of the mPT [7,18,19,56,57] and appear to act by direct competition with Ca^{2+} for its binding at the trigger site. Bernardi and colleagues have presented data to show that the effect of low pH involves a specific histidine residue and that this may modulate CyP binding to the IMM [26,32]. However, in our experiments we did not detect such an effect of low pH on CyP binding to either inner membranes [18] or to the purified ANT [48]. Trifluoperazine is a potent inhibitor of

the mPT under energized but not de-energized conditions [19]. It was originally thought to act indirectly through inhibition of phospholipase A_2, preventing the accumulation of free fatty acids which stimulate the mPT, probably through interaction with the ANT [10,58]. However, inhibition occurs even without changes in free fatty acid accumulation and is now thought to be mediated by an effect on surface membrane charge that changes the voltage sensitivity of the mPT [59]. Very recently it has been suggested that in muscle mitochondria the rate of electron flow through complex 1 of the respiratory chain may exert a regulatory effect on the mPT, but the mechanism by which this is mediated remains unclear [60].

The role of the mPT in reperfusion injury of the heart

When ischaemic tissues are reperfused, the damage caused during anoxia is further exacerbated. This phenomenon, known as reperfusion injury, has been widely studied (see [2,4,61–64]) and the mechanisms thought to be involved are summarized in Figure 2. During the ischaemic phase, cells endeavour, unsuccessfully, to maintain their ATP levels through glycolysis, which leads to an accumulation of lactic acid and a decrease in intracellular pH (pH_i) [65]. The Na^+/H^+ antiporter is activated in an attempt to restore pH_i [66,67], but this loads the cell with Na^+, which cannot be pumped out again through the operation of the Na^+/K^+-ATPase if there is insufficient ATP to drive the process [68]. The accumulated Na^+ prevents Ca^{2+} from being pumped out of the cell on the Na^+/Ca^{2+} antiporter and may actually reverse the process, allowing additional Ca^{2+} to enter the cytosol from the plasma [68,69]. Some of this calcium may also enter the mitochondria by reversal of the Na^+/Ca^{2+} antiporter [70], but upon reperfusion Ca^{2+} is rapidly taken up into the mitochondria by means of the uniporter, loading the mitochondrial matrix with large amounts of Ca^{2+} [71,72]. Alone, this might not be sufficient to activate the mPT, but other factors also come into play during reperfusion. The sudden influx of oxygen into the anoxic cell induces the formation of oxygen free radicals through an interaction of oxygen with ubisemiquinone. This accumulates during anoxia as a result of respiratory chain inhibition [2,61,73,74]. Additional oxygen free radicals may be produced through the operation of xanthine oxidase [75]. This enzyme is activated during hypoxia and is presented with high concentrations of xanthine produced by the purine degradation that occurs during ischaemia [76,76a]. The combination of oxidative stress and high $[Ca^{2+}]$ provides the ideal conditions for the mPT, especially in the presence of elevated cellular phosphate concentrations and depleted adenine nucleotide levels, both of which occur during the ischaemic phase [2,61,68]. Furthermore, during the reperfusion phase the pH_i rapidly returns to pre-ischaemic values through the operation of the Na^+/H^+ antiporter, lactic acid efflux on the monocarboxylate transporter and bicarbonate-dependent mechanisms [67]. It will be recalled that low pH (<7.0) exerts a powerful inhibitory effect on the mPT [7,56,57], and when this is removed on reperfusion, the factors that are in place to stimulate the mPT can now exert their full effect.

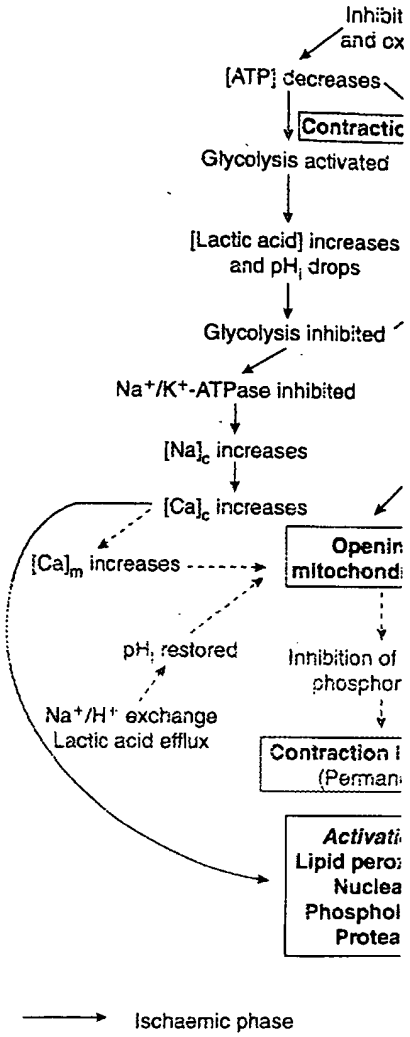

Figure 2 Scheme summarizi reperfusion injury. $[Ca]_m$, matrix and $[Ca^{2+}]$, respectively.

The sequence of events descri central in determining whether a cel reperfusion. Indeed, morphologica swollen and amorphous under such

:ed conditions [19]. It was origi-
nhibition of phospholipase A_2,
s which stimulate the mPT, prob-
58]. However, inhibition occurs
ulation and is now thought to be
charge that changes the voltage
ıas been suggested that in muscle
ıgh complex 1 of the respiratory
PT, but the mechanism by which

injury of the heart

the damage caused during anoxia
nown as reperfusion injury, has
the mechanisms thought to be
:he ischaemic phase, cells endeav-
levels through glycolysis, which
lecrease in intracellular pH (pH_i)
n attempt to restore pH_i [66,67],
ot be pumped out again through
e is insufficient ATP to drive the
Ca^{2+} from being pumped out of
ay actually reverse the process,
from the plasma [68,69]. Some of
ia by reversal of the Na^+/Ca^{2+}
rapidly taken up into the mito-
the mitochondrial matrix with
ight not be sufficient to activate
during reperfusion. The sudden
ıe formation of oxygen free radi-
oisemiquinone. This accumulates
ıhibition [2,61,73,74]. Additional
h the operation of xanthine oxi-
poxia and is presented with high
purine degradation that occurs
f oxidative stress and high $[Ca^{2+}]$
ecially in the presence of elevated
d adenine nucleotide levels, both
,61,68]. Furthermore, during the
ore-ischaemic values through the
d efflux on the monocarboxylate
ıanisms [67]. It will be recalled
ory effect on the mPT [7,56,57],
factors that are in place to stimu-

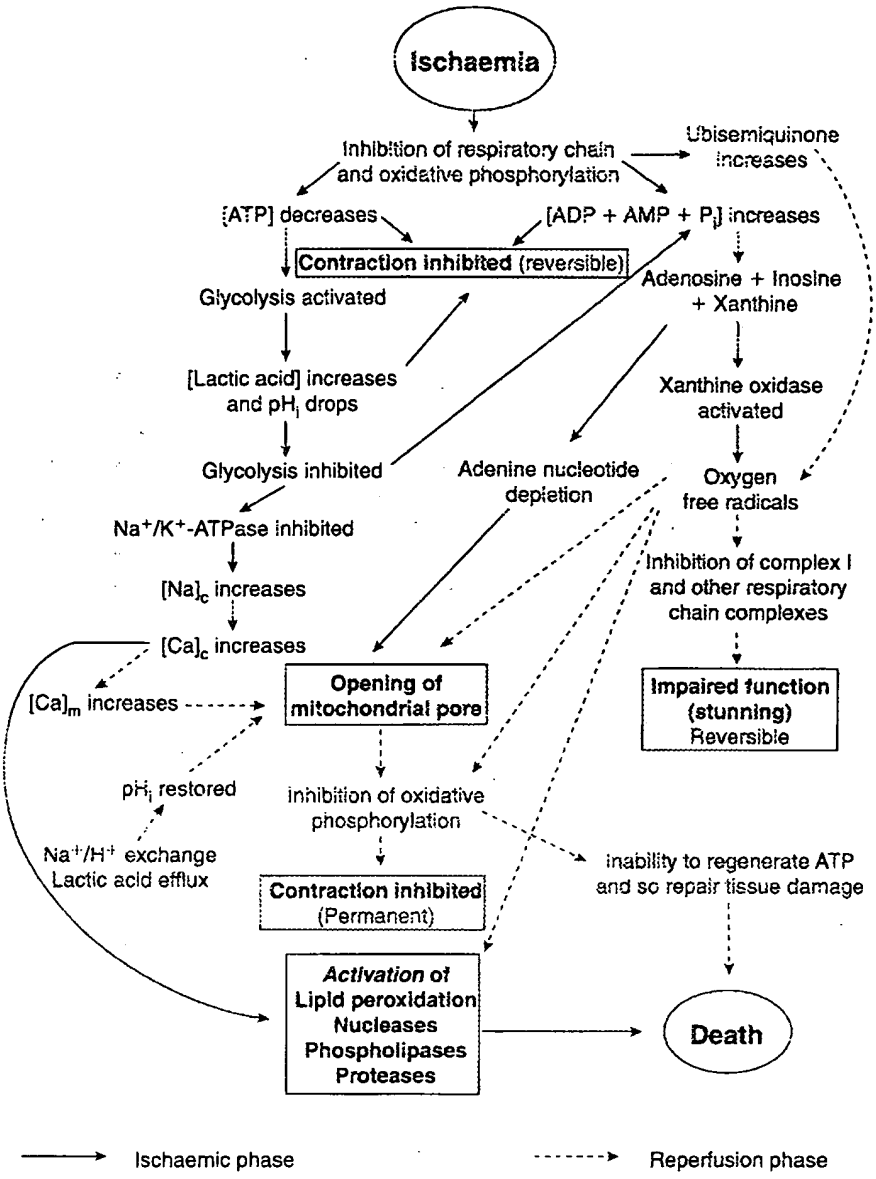

Figure 2 Scheme summarizing the main events that occur in reperfusion injury. $[Ca]_m$, matrix $[Ca^{2+}]$; $[Na]_c$ and $[Ca]_c$, cytoplasmic $[Na^+]$ and $[Ca^{2+}]$, respectively.

The sequence of events described earlier suggests that the mPT may be central in determining whether a cell lives or dies in response to ischaemia and reperfusion. Indeed, morphological studies confirm mitochondria become swollen and amorphous under such conditions [2,62]. If the mPT is a critical

factor in the development of reperfusion injury, CsA would be expected to provide some protection from damage. This has been observed in isolated cardiac myocytes and hepatocytes subjected to re-oxygenation following a period of hypoxia [77,78], in hepatocytes during chemical anoxia and oxidative stress [79–81], in the brain following a hypoglycaemic insult [82] and in perfused livers and hearts subjected to isothermic global ischaemia followed by reperfusion [24,64,83–85]. In the latter case we demonstrated that hearts treated with 0.2 μM CsA showed a greater recovery of left ventricular developed pressure (LVDP), tissue ATP/ADP ratios and functional mitochondria, whereas AMP levels and end-diastolic pressure (an indicator of contracture due to low ATP/ADP and elevated $[Ca^{2+}]$) were lower. No protective effect of CsA was observed on the loss of total adenine nucleotides that occurs as a result of purine degradation during hypoxia. Nor was protection from inhibition of respiratory chain function (state-3 substrate oxidation) observed [24,64,83]. The latter effect is probably caused by oxygen free radicals, formed during ischaemia and reperfusion, directly modifying components of the respiratory chain [24,61,64,83]. Our observations are consistent with CsA exerting its effects by inhibiting the mPT, which is downstream of changes in total adenine nucleotides and free radicals. Furthermore, we have demonstrated that only analogues of CsA that block the mPT in isolated mitochondria are able to offer protection to the reperfused heart [24,64]. No protection is exerted by cyclosporin H and FK506 (another drug whose immunosuppressive action, like that of CsA, is exerted through inhibition of calcineurin), neither of which bind to CyP-D [24,83]. The protective effect of CsA was highly concentration dependent, showing an optimal response at 0.2 μM and declining at higher concentrations [83]. A similar concentration dependence has been observed for CsA protection of isolated cardiac myocytes subjected to re-oxygenation following a period of hypoxia [77].

Direct measurement of pore opening during reperfusion of the ischaemic heart

To establish directly that reperfusion injury of the heart is associated with the mPT it is necessary to measure mitochondrial pore opening *in situ*. Techniques involving the use of fluorescent probes that can easily be applied to isolated cells [80,86] are not appropriate for the perfused heart and we have devised an alternative procedure that relies on the impermeability of the IMM to 2-deoxyglucose 6-phosphate [24,64]. Hearts are perfused in Langendorff recirculating mode with [^{3}H]2-deoxyglucose (DOG), which enters the heart on the glucose carrier and is then phosphorylated to DOG 6-phosphate without further metabolism. Thus [^{3}H]DOG 6-phosphate is trapped in the cytosol of the heart cell, and only enters the mitochondria if the pore opens. Extracellular [^{3}H]DOG is removed form the heart by perfusion in the absence of [^{3}H]DOG, before hearts are subjected to various periods of ischaemia and reperfusion. Mitochondria are then prepared rapidly and assayed for [^{3}H]DOG and citrate synthase (an indicator of mitochondrial recovery). From the [^{3}H]DOG content of the mitochondria and a small sample of total heart homogenate an estimation of pore opening is possible. This technique allowed

us to demonstrate that mitochond
period had not taken up DOG 6-
a period of reperfusion showed a s
[24]. Maximal mitochondrial DO
sion [64], the same time period
pre-ischaemic values [67,87]. Thus
the mitochondrial pore occurs on
with the predictions made earlier.
primary cause of cell injury but
following other critical damage t
plasma membrane permeability b
occur, DOG would be lost from t
and thus no increase in mitochond

Reversal of the mPT in hearts t

The extent of functional rec
quantified by using pressure trans
pressure and aortic pressure. In
can be determined in hearts freeze
reperfusion [83]. Reperfusion aft
recovery of LVDP and ATP/ADI
DOG entrapment is still observed,
even though the heart recovers f
chondrial pores open in the early
allowing total recovery of mitoc
Unfortunately, when the mPT r
means that DOG remains trapped
not detected using our normal pro
modified to determine whether re
are loaded with [^{3}H]DOG after m
lished during reperfusion. If mit
DOG entrapment determined usi
than when DOG is present at the
confirmed that this is the case [4,8
about 50% less mitochondrial DC
ing [4,87]. Thus it would seem
reperfusion is not too great, mitoc
by closure of the pores and entrap
brought about by the decrease in
from the mitochondria during the
cals by superoxide dismutase and
only occur if enough 'healthy' mi
mulate the released calcium and
maintain the ionic and reduced g
ance between the number of 'clos
will be critical in determining whe
'open' mitochondria, they will re

njury, CsA would be expected to
; has been observed in isolated car-
. re-oxygenation following a period
hemical anoxia and oxidative stress
emic insult [82] and in perfused liv-
] ischaemia followed by reperfusion
onstrated that hearts treated with
left ventricular developed pressure
tional mitochondria, whereas AMP
icator of contracture due to low
r. No protective effect of CsA was
leotides that occurs as a result of
as protection from inhibition of res-
oxidation) observed [24,64,83]. The
gen free radicals, formed during
ying components of the respiratory
consistent with CsA exerting its
vnstream of changes in total adenine
e, we have demonstrated that only
olated mitochondria are able to offer
64]. No protection is exerted by
whose immunosuppressive action,
ion of calcineurin), neither of which
ect of CsA was highly concentration
at 0.2 μM and declining at higher
n dependence has been observed for
tes subjected to re-oxygenation fol-

during reperfusion of the

injury of the heart is associated with
itochondrial pore opening *in situ*.
nt probes that can easily be applied to
for the perfused heart and we have
s on the impermeability of the IMM
Hearts are perfused in Langendorff
cose (DOG), which enters the heart
norylated to DOG 6-phosphate with-
6-phosphate is trapped in the cytosol
e mitochondria if the pore opens.
the heart by perfusion in the absence
d to various periods of ischaemia and
prepared rapidly and assayed for
tor of mitochondrial recovery). From
dria and a small sample of total heart
ng is possible. This technique allowed

us to demonstrate that mitochondria prepared immediately after the ischaemic period had not taken up DOG 6-phosphate whereas those prepared following a period of reperfusion showed a significant uptake, indicative of pore opening [24]. Maximal mitochondrial DOG uptake was found after 5 min of reperfusion [64], the same time period over which the intracellular pH returns to pre-ischaemic values [67,87]. Thus our data suggest that a profound opening of the mitochondrial pore occurs only during the reperfusion phase, consistent with the predictions made earlier. It has been argued that pore opening is not a primary cause of cell injury but rather a secondary phenomenon that occurs following other critical damage to the myocyte, such as breakdown of the plasma membrane permeability barrier [88]. However, if the latter were to occur, DOG would be lost from the cell before it could enter the mitochondria and thus no increase in mitochondrial DOG would be measured.

Reversal of the mPT in hearts that recover during reperfusion

The extent of functional recovery of the heart during reperfusion can be quantified by using pressure transducers to monitor beat, LVDP, end-diastolic pressure and aortic pressure. In addition, adenine nucleotide concentrations can be determined in hearts freeze–clamped after the ischaemic period or after reperfusion [83]. Reperfusion after short periods of ischaemia leads to total recovery of LVDP and ATP/ADP ratio [83], yet an increase in mitochondrial DOG entrapment is still observed, indicating that the mPT must have occurred even though the heart recovers fully [24,64,83]. This suggests that the mitochondrial pores open in the early phase of reperfusion but then rapidly reseal, allowing total recovery of mitochondrial function and heart performance. Unfortunately, when the mPT reverses, closure of the mitochondrial pores means that DOG remains trapped inside the mitochondria and the reversal is not detected using our normal protocol. However, the DOG technique can be modified to determine whether resealing has occurred. For this purpose, hearts are loaded with [^{3}H]DOG after maximum recovery of the heart has been established during reperfusion. If mitochondrial pores close during reperfusion, DOG entrapment determined using this 'post-loading' protocol should be less than when DOG is present at the start of reperfusion (pre-loading). We have confirmed that this is the case [4,87]. After 40 min ischaemia post-loading gives about 50% less mitochondrial DOG entrapment than observed with pre-loading [4,87]. Thus it would seem that if the insult caused by ischaemia/reperfusion is not too great, mitochondria can undergo a transient PT, followed by closure of the pores and entrapment of the DOG. The closure is presumably brought about by the decrease in matrix [Ca^{2+}] that occurs as calcium is lost from the mitochondria during the mPT, and the removal of oxygen free radicals by superoxide dismutase and glutathione peroxidase. However, this will only occur if enough 'healthy' mitochondria are remaining in the cell to accumulate the released calcium and provide sufficient ATP and NADPH to maintain the ionic and reduced glutathione homoeostasis of the cell. The balance between the number of 'closed' and 'open' mitochondria within any cell will be critical in determining whether a cell lives or dies. If there are too many 'open' mitochondria, they will release more calcium and hydrolyse more ATP

than the 'closed' mitochondria can compensate for. In contrast, if there are sufficient 'closed' mitochondria to meet the cell's ATP requirements and to accumulate released calcium without undergoing the mPT themselves, the 'open' mitochondria will close again and the cell will recover.

Strategies for inhibiting the mPT that can be used to protect the heart from reperfusion injury

Although we have demonstrated that CsA can be used effectively to protect the perfused heart from reperfusion injury, the degree of protection was very sensitive to the concentration of CsA used and to the conditions used for the perfusion ([24,64,83] and P.M. Kerr and A.P. Halestrap, unpublished work). In some situations CsA was actually found to impair recovery of the heart from ischaemia and thus it is unlikely to be appropriate for use in open heart surgery. Consequently, we have investigated the use of other means to inhibit the mPT during reperfusion.

Anti-oxidants and calcium antagonists

There is an extensive literature to show that anti-oxidants and free-radical scavengers can protect the ischaemic/reperfused heart from irreversible damage (see [61,88]). Whereas there are many processes within the cardiac myocyte that are possible targets for the action of these reagents, prevention of the mPT is clearly one of them. Evidence is also strong that protection from reperfusion injury can be achieved by preventing the mitochondrial calcium overload accompanying reperfusion through the use of calcium antagonists or Ruthenium Red, an inhibitor of mitochondrial calcium uptake [71,89–97]. These data are consistent with inhibition of the mPT that such reagents would be predicted to cause.

Low pH_i

It is well established that low pH_i (<7.0), induced by the use of low extracellular pH or by addition of specific inhibitors of the Na^+/H^+ antiporter such as amiloride, can protect a variety of cells, including cardiac myocytes and hepatocytes, from oxidative stress, re-oxygenation following anoxia or reperfusion following ischaemia [78,98–106]. Although low pH_i may have several means of exerting its protective effects, the profound inhibition of the mPT at pH<7.0 is likely to be an important one and has been demonstrated directly in hepatocytes [78]. Furthermore, in the ischaemic/reperfused heart, mitochondrial pores open over the same period of time as the pH_i takes to be restored from <6.5 to pre-ischaemic values (>7.0) [64,67,87].

Pyruvate

It is well documented that pyruvate can protect hearts [107–109] and other tissues [110–112] from ischaemia/reperfusion and anoxia/re-oxygenation injury. The protective effects of pyruvate have been attributed to beneficial metabolic alterations and to protection from free-radical production, since pyruvate acts as a free-radical scavenger [109,110]. However, an additional effect of pyruvate might be through inhibition of the mPT, in part through its

free-radical scavenging effects. In
pyruvate might increase both the
would inhibit the mPT, and the N
oxidation of protein thiol groups c
Furthermore, pyruvate is transport
of the monocarboxylate transporter
pH_i directly. In parallel, competitic
port by the monocarboxylate tra
intracellular accumulation of lactic a
ing additional inhibition of the mP
perfusate pH of pyruvate-treated he
than for control hearts. This implies
in pH_i both at the end of ischaemia
Indeed, there is also direct evidenc
that pyruvate causes a decrease in p
Using the DOG technique we ha
10 mM pyruvate (present both befo
nied by a reduction of mitochondri
reperfusion and more extensive po
the presence of pyruvate, hearts rec
ischaemia compared with only abo
is associated with DOG entrapm
opposed to only a 50% decrease in
These data are the first direct evid
reverse fully when hearts recover th

Propofol

Propofol is an anaesthetic tha
and in post-operative sedation [120
a free-radical scavenger [121,122] a
those used clinically, it may inhibit
Other studies have indicated that pr
sion injury and hydrogen peroxide-
heart [125–127]. In our own studies
a concentration similar to that en
ischaemia and during reperfusion c
hearts from reperfusion injury (S. J
lished work). Recovery of LVDP
increased from 36 ± 8% (n = 10) in
in propofol-treated hearts. This wa
chondrial DOG entrapment. H
mitochondria at the same concen
against the mPT, suggesting that its
may not be through a direct effect
may be secondary to propofol's r
through inhibitory effects on calci
radical scavenger [121,122]. Nevert

te for. In contrast, if there are suf-
cell's ATP requirements and to
going the mPT themselves, the
ell will recover.

be used to protect the heart

sA can be used effectively to pro-
ıry, the degree of protection was
ed and to the conditions used for
nd A.P. Halestrap, unpublished
found to impair recovery of the
:o be appropriate for use in open
igated the use of other means to

hat anti-oxidants and free-radical
ed heart from irreversible damage
sses within the cardiac myocyte
: reagents, prevention of the mPT
that protection from reperfusion
mitochondrial calcium overload
ıse of calcium antagonists or
drial calcium uptake [71,89–97].
ıe mPT that such reagents would

, induced by the use of low extra-
rs of the Na^+/H^+ antiporter such
including cardiac myocytes and
ıation following anoxia or reper-
ough low pH_i may have several
ofound inhibition of the mPT at
ıas been demonstrated directly in
ic/reperfused heart, mitochondr-
the pH_i takes to be restored from

an protect hearts [107–109] and
usion and anoxia/re-oxygenation
ıve been attributed to beneficial
n free-radical production, since
9,110]. However, an additional
ı of the mPT, in part through its

free-radical scavenging effects. In addition, as a good respiratory substrate pyruvate might increase both the mitochondrial membrane potential, which would inhibit the mPT, and the NADH/NAD^+, which would help prevent oxidation of protein thiol groups critical for activation of the mPT [113–115]. Furthermore, pyruvate is transported into heart cells with a proton by means of the monocarboxylate transporter [65,116], which might lead to a decrease in pH_i directly. In parallel, competition between pyruvate and lactate for transport by the monocarboxylate transporter [65,116] may lead to a greater intracellular accumulation of lactic acid, further lowering of pH_i and thus causing additional inhibition of the mPT. We have demonstrated that the drop in perfusate pH of pyruvate-treated hearts on reperfusion is considerably greater than for control hearts. This implies that pyruvate causes a significant decrease in pH_i both at the end of ischaemia and during the reperfusion phase [87,117]. Indeed, there is also direct evidence from nuclear magnetic resonance studies that pyruvate causes a decrease in pH_i in a low-flow model of ischaemia [118]. Using the DOG technique we have confirmed that the protective effect of 10 mM pyruvate (present both before, during and after ischaemia) is accompanied by a reduction of mitochondrial pore opening during the initial stages of reperfusion and more extensive pore closure at later stages [87,119]. Thus, in the presence of pyruvate, hearts recover 100% of their LVDP after 40 min of ischaemia compared with only about 50% in the absence of pyruvate, and this is associated with DOG entrapment returning to pre-ischaemic values as opposed to only a 50% decrease in DOG entrapment in controls [87,117,119]. These data are the first direct evidence that mitochondrial pore opening can reverse fully when hearts recover their function during reperfusion.

Propofol

Propofol is an anaesthetic that is frequently used during cardiac surgery and in post-operative sedation [120]. There are reports that propofol can act as a free-radical scavenger [121,122] and also that, at concentrations higher than those used clinically, it may inhibit the PT of isolated mitochondria [123,124]. Other studies have indicated that propofol can attenuate the effects of reperfusion injury and hydrogen peroxide-induced oxidative stress of the perfused rat heart [125–127]. In our own studies we have confirmed that 2 μg/ml propofol, a concentration similar to that employed clinically, added 10 min prior to ischaemia and during reperfusion causes significant protection of isolated rat hearts from reperfusion injury (S. Javadov, P. Kerr and A. Halestrap, unpublished work). Recovery of LVDP after 30 min of ischaemia (means ± S.E.M.) increased from 36 ± 8% ($n = 10$) in control hearts to 70 ± 11% ($n = 8$; $P<0.05$) in propofol-treated hearts. This was accompanied by a 25% decrease in mitochondrial DOG entrapment. However, when added to isolated heart mitochondria at the same concentration, propofol afforded no protection against the mPT, suggesting that its protective effect against reperfusion injury may not be through a direct effect on the mPT. Rather, inhibition of the mPT may be secondary to propofol's reported abilities to lower cytosolic $[Ca^{2+}]$ through inhibitory effects on calcium channels [128,129] and to act as a free-radical scavenger [121,122]. Nevertheless, propofol provides another example

of a reagent whose protection of the heart from reperfusion injury is accompanied by a decrease in mitochondrial pore opening *in vivo*.

Preconditioning

Hearts subjected to two or three brief (3–5 min) ischaemic periods with intervening recovery periods before a prolonged period of ischaemia experience substantial protection against reperfusion injury [130,131]. It is thought that a major player in the mechanism of preconditioning is receptor-mediated activation of protein kinase C that occurs in response to the release of mediators, such as adenosine, bradykinin, endothelin-1, opioids and catecholamines, released during the brief ischaemic periods [130–134]. The means by which protein kinase C exerts its protective effects is not firmly established but a role for activation of K_{ATP} channels has been suggested since preconditioning is prevented by sulphonylureas, which are potent inhibitors of the K_{ATP} channel [135–137]. Whatever the mechanism of preconditioning, our own experiments using the DOG entrapment technique did not detect any decrease in mitochondrial pore opening (P.M. Kerr and A.P. Halestrap, unpublished work), although others have reported that mitochondrial damage is reduced in preconditioned hearts [138]. Thus protection of hearts and their mitochondria can occur without inhibition of the mPT. One observation that might account for how this is achieved is that the mitochondrial ATPase inhibitor protein is activated during the brief ischaemic periods [139,140]. The resulting inhibition of the ATPase may prevent 'open' mitochondria from degrading the ATP generated by glycolysis and the remaining 'closed' mitochondria. As a result, hearts would remain protected from reperfusion injury even when a significant number of mitochondria are in an 'open' state.

The mPT and apoptosis in the heart

Some myocytes in the failing heart undergo apoptotic cell death, as do myocytes in areas surrounding a myocardial infarct, i.e. areas that experience a less-pronounced ischaemic insult than that which leads to necrosis [141–147]. Recent evidence suggests that the mPT may act as the 'central executioner' of cells subjected to a range of insults, such as oxidative stress, growth factor removal or exposure to cytokines. Indeed, the mitochondria may not only determine whether a cell lives or dies, but also whether death occurs by apoptosis or necrosis [52,148–150]. Thus in some cells changes in $\Delta\Psi_m$ occur during early stages of apoptosis and can be inhibited by CsA, which also inhibits apoptosis [151,152]. Furthermore, mitochondria are required to induce apoptosis in a cell-free system, which they do by releasing apoptosis-inducing factors, the best characterized of which is cytochrome *c* [150,153–155]. In fact, work in this laboratory established many years ago that cytochrome *c* release does occur during the mPT [156]. The anti-apoptotic gene product, Bcl-2, is associated with the mitochondrial outer membrane and has been reported to inhibit the mPT and prevent release of cytochrome *c* and consequent caspase activation [157–159]. Attractive though this hypothesis may be, in many situa-

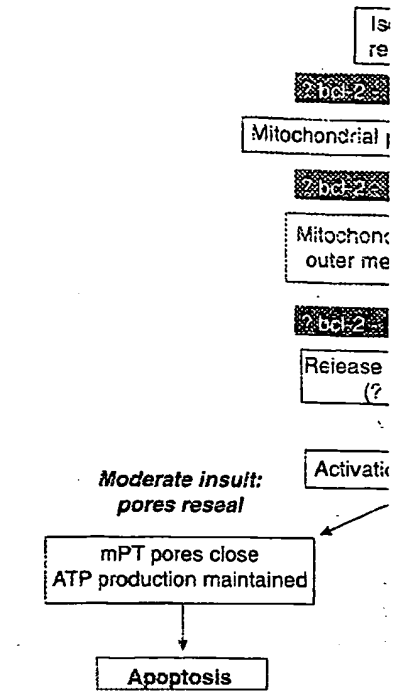

Figure 3 Scheme illustrating
deciding whether a cell dies by
inducing factor.

tions apoptosis can occur without e
does CsA protect every cell type fr
induce apoptosis under some circun
seem likely that cells experiencing on
opening of the mPT pore which the
ATP production to be re-establis
swelling of the outer membrane may
motion the apoptotic cascade that ca
death by apoptosis that can be attent
a clear example of this is the apoptot
pus 24 h after a 30 min period
However, the controlled nature of a
is maintained, and where this is n
[168,169]. Such a situation appears t
where mPT pore opening is both ext
tions cytochrome *c* will be released,
unable to generate the ATP required
sis. Neither can tissue damage be
ultimately leading to rupture of th
uncontrolled necrotic form of cell d
bated as neutrophil invasion leads

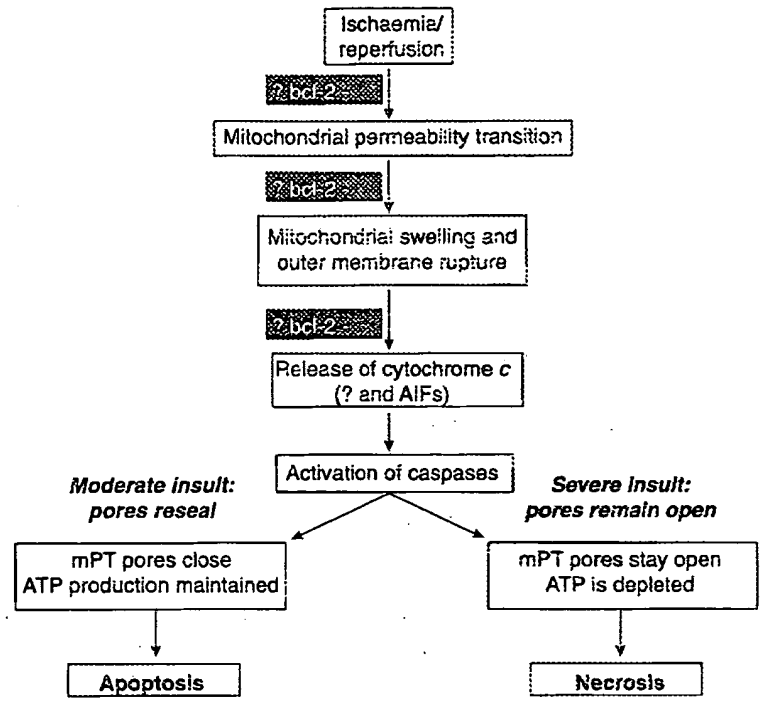

Figure 3 Scheme illustrating how the mPT may be involved in deciding whether a cell dies by necrosis or apoptosis. AIF, apoptosis-inducing factor.

tions apoptosis can occur without early changes in $\Delta\Psi_m$ [157,160–164]. Nor does CsA protect every cell type from all apoptotic stimuli, and it may even induce apoptosis under some circumstances [160,164–167]. However, it does seem likely that cells experiencing only a modest insult may undergo a transient opening of the mPT pore which then closes again, enabling ion gradients and ATP production to be re-established. Under such conditions, sufficient swelling of the outer membrane may occur to release cytochrome *c* and set in motion the apoptotic cascade that causes an organized, non-inflammatory cell death by apoptosis that can be attenuated by CsA. We have demonstrated that a clear example of this is the apoptotic cell death that is seen in the hippocampus 24 h after a 30 min period of insulin-induced hypoglycaemia [82]. However, the controlled nature of apoptosis requires that tissue ATP content is maintained, and where this is not the case cell death becomes necrotic [168,169]. Such a situation appears to occur in irreversible reperfusion injury where mPT pore opening is both extensive and prolonged. Under these conditions cytochrome *c* will be released, but mitochondria remain uncoupled and unable to generate the ATP required for maintaining cellular ionic homoeostasis. Neither can tissue damage be repaired and it will continue unabated, ultimately leading to rupture of the plasma membrane and cell death. This uncontrolled necrotic form of cell death is inflammatory and further exacerbated as neutrophil invasion leads to yet more damage. Thus the decision

between apoptosis and necrosis may rest on the extent of the mPT and can account for the observation that apoptosis and necrosis both occur in the reperfused heart, with the least damaged areas showing a preponderance of apoptosis over necrosis. A diagram summarizing how the mPT may act as the decision maker between apoptosis and necrosis is given in Figure 3.

Conclusions

The mPT converts the mitochondrion from an organelle that, through its production of ATP, sustains the cell in its normal function to an instrument of death. There is now strong evidence that the mPT involves a calcium-mediated conformational change in the ANT that converts it into a non-specific pore (see Figure 1). Except at very high [Ca^{2+}], mitochondrial CyP is required to implement this conformational change and the process is further sensitized to [Ca^{2+}] by oxidative stress. In contrast, decreasing pH_i below 7.0 greatly desensitizes the mPT to [Ca^{2+}]. This information can be used to devise strategies that may protect tissues such as the heart and brain from damage caused by ischaemia and reperfusion injury and thus has important clinical implications.

This work was supported by project grants from the Medical Research Council and the British Heart Foundation.

References

1. Bernardi, P., Broekemeier, K.M. and Pfeiffer, D.R. (1994) J. Bioenerg. Biomembr. 26, 509–517
2. Halestrap, A.P. (1994) In Mitochondria: DNA, proteins and disease (Darley-Usmar, V. and Schapira, A.H.V., eds.), pp. 113–142, Portland Press Ltd, London
3. Zoratti, M. and Szabo, I. (1995) Biochim. Biophys. Acta 1241, 139–176
4. Halestrap, A.P., Kerr, P.M., Javadov, S. and Woodfield, K.-Y. (1998) Biochim. Biophys. Acta, in the press
5. Chappell, J.B. and Crofts, A.R. (1965) Biochem. J. 95, 378–386
6. Hunter, D.R. and Haworth, R.A. (1979) Arch. Biochem. Biophys. 195, 453–459
7. Haworth, R.A. and Hunter, D.R. (1979) Arch. Biochem. Biophys. 195, 460–467
8. Al-Nasser, I. and Crompton, M. (1986) Biochem. J. 239, 19–29
9. Crompton, M., Costi, A. and Hayat, L. (1987) Biochem. J. 245, 915–918
10. Gunter, T.E. and Pfeiffer, D.R. (1990) Am. J. Physiol. 258, C755–C786
11. Crompton, M., Ellinger, H. and Costi, A. (1988) Biochem. J. 255, 357–360
12. Broekemeier, K.M., Dempsey, M.E. and Pfeiffer, D.R. (1989) J. Biol. Chem. 264, 7826–7830
13. Halestrap, A.P. and Davidson, A.M. (1990) Biochem. J. 268, 153–160
14. Galat, A. and Metcalfe, S.M. (1995) Prog. Biophys. Mol. Biol. 63, 67–118
15. Crompton, M. and Costi, A. (1988) Eur. J. Biochem. 178, 489–501
16. Crompton, M. and Costi, A. (1990) Biochem. J. 266, 33–39
17. Connern, C.P. and Halestrap, A.P. (1994) Biochem. J. 302, 321–324
18. Connern, C.P. and Halestrap, A.P. (1996) Biochemistry 35, 8175–8180
19. Halestrap, A.P., Woodfield, K.-Y. and Connern, C.P. (1996) J. Biol. Chem. 272, 3346–3354
20. Petronilli, V., Szabo, I. and Zoratti, M. (1989) FEBS Lett. 259, 137–143
21. Ichas, F., Jouaville, L.S. and Mazat, J.P. (1997) Cell 89, 1145–1153

22. Novgorodov, S.A., Gudz, T.I., Milgron 16274–16282
23. Crompton, M. and Andreeva, L. (1994)
24. Griffiths, E.J. and Halestrap, A.P. (1995
25. Griffiths, E.J. and Halestrap, A.P. (199
26. Nicolli, A., Basso, E., Petronilli, V., We 271, 2185–2192
27. Connern, C.P. and Halestrap, A.P. (199
28. Woodfield, K.Y., Price, N.T. and Halest 27–30
29. Bergsma, D.J., Eder, C., Gross, M., Ker D., Livi, G.P., Mclaughlin, M.M., Kasya
30. Andreeva, L. and Crompton, M. (1994)
31. Andreeva, L., Tanveer, A. and Crompto
32. Nicolli, A., Petronilli, V. and Bernardi, I
33. LeQuoc, K. and LeQuoc, D. (1988) Arc
34. Novgorodov, S.A., Gudz, T.I., Brierley, Biophys. 311, 219–228
35. Majima, E., Koike, H., Hong, Y.M., Shi 22181–22187
36. Majima, E., Yamaguchi, N., Chuman, H (1998) Biochemistry 37, 424–432
37. Bernardi, P. (1992) J. Biol. Chem. 267, 8
38. Petronilli, V., Cola, C. and Bernardi, P.
39. Petronilli, V., Costantini, P., Scorrano, I (1994) J. Biol. Chem. 269, 16638–16642
40. Scorrano, L., Petronilli, V. and Bernardi,
41. Soverijn, J.H.M., Huisman, L.A., Rosing 305, 185–198
42. Klingenberg, M. (1980) J. Membr. Biol.
43. Krämer, R. and Klingenberg, M. (1982) I
44. Costantini, P., Chernyak, B.V., Petronili 6746–6751
45. Chernyak, B.V. and Bernardi, P. (1996) I
46. Eriksson, O., Fontaine, E., Petronilli, V.
47. Eriksson, O., Fontaine, E. and Bernardi,
48. Woodfield, K., Rück, A., Brdiczka, D. a
49. Brustovetsky, N. and Klingenberg, M. (1
50. Rück, A., Dolder, M., Wallimann, T. and
51. Halestrap, A.P. (1987) Biochem. J. 244, 1
52. Zamzami, N., Brenner, C., Marzo, I., Su 2265–2282
53. Kroemer, G., Dallaporta, B. and Resche
54. Beutner, G., Ruck, A., Riede, B., Welte, 189–195
55. McEnery, M.W., Snowman, A.M., Trifil Sci. U.S.A. 89, 3170–3174
56. Halestrap, A.P. (1991) Biochem. J. 278, 7
57. Bernardi, P., Vassanelli, S., Veronese, P., J. Biol. Chem. 267, 2934–2939
58. Broekemeier, K.M., Schmid, P.C., Schmi 260, 105–113

ı the extent of the mPT and can
l necrosis both occur in the reper-
s showing a preponderance of
:ing how the mPT may act as the
is is given in Figure 3.

:om an organelle that, through its
:mal function to an instrument of
nPT involves a calcium-mediated
:rts it into a non-specific pore (see
ondrial CyP is required to imple-
:ess is further sensitized to $[Ca^{2+}]$
H_i below 7.0 greatly desensitizes
ısed to devise strategies that may
om damage caused by ischaemia
clinical implications.

the Medical Research Council and

(1994) J. Bioenerg. Biomembr. 26,

ɔteins and disease (Darley-Usmar, V. and
is Ltd, London
Acta 1241, 139–176
field, K.-Y. (1998) Biochim. Biophys.

95, 378–386
chem. Biophys. 195, 453–459
chem. Biophys. 195, 460–467
. 239, 19–29
:hem. J. 245, 915–918
iol. 258, C755–C786
iochem. J. 255, 357–360
).R. (1989) J. Biol. Chem. 264, 7826–7830
m. J. 268, 153–160
Mol. Biol. 63, 67–118
n. 178, 489–501
6, 33–39
ı. J. 302, 321–324
iistry 35, 8175–8180
:.P. (1996) J. Biol. Chem. 272, 3346–3354
S Lett. 259, 137–143
89, 1145–1153

22. Novgorodov, S.A., Gudz, T.I., Milgrom, Y.M. and Brierley, G.P. (1992) J. Biol. Chem. 267, 16274–16282
23. Crompton, M. and Andreeva, L. (1994) Biochem. J. 302, 181–185
24. Griffiths, E.J. and Halestrap, A.P. (1995) Biochem. J. 307, 93–98
25. Griffiths, E.J. and Halestrap, A.P. (1991) Biochem. J. 274, 611–614
26. Nicolli, A., Basso, E., Petronilli, V., Wenger, R.M. and Bernardi, P. (1996) J. Biol. Chem. 271, 2185–2192
27. Connern, C.P. and Halestrap, A.P. (1992) Biochem. J. 284, 381–385
28. Woodfield, K.Y., Price, N.T. and Halestrap, A.P. (1997) Biochim. Biophys. Acta 1351, 27–30
29. Bergsma, D.J., Eder, C., Gross, M., Kersten, H., Sylvester, D., Appelbaum, E., Cusimano, D., Livi, G.P., Mclaughlin, M.M., Kasyan, K. et al. (1991) J. Biol. Chem. 266, 23204–23214
30. Andreeva, L. and Crompton, M. (1994) Eur. J. Biochem. 221, 261–268
31. Andreeva, L., Tanveer, A. and Crompton, M. (1995) Eur. J. Biochem. 230, 1125–1132
32. Nicolli, A., Petronilli, V. and Bernardi, P. (1993) Biochemistry 32, 4461–4465
33. LeQuoc, K. and LeQuoc, D. (1988) Arch. Biochem. Biophys. 265, 249–257
34. Novgorodov, S.A., Gudz, T.I., Brierley, G.P. and Pfeiffer, D.R. (1994) Arch. Biochem. Biophys. 311, 219–228
35. Majima, E., Koike, H., Hong, Y.M., Shinohara, Y. and Terada, H. (1993) J. Biol. Chem. 268, 22181–22187
36. Majima, E., Yamaguchi, N., Chuman, H., Shinohara, Y., Ishida, M., Goto, S. and Terada, H. (1998) Biochemistry 37, 424–432
37. Bernardi, P. (1992) J. Biol. Chem. 267, 8834–8839
38. Petronilli, V., Cola, C. and Bernardi, P. (1993) J. Biol. Chem. 268, 1011–1016
39. Petronilli, V., Costantini, P., Scorrano, L., Colonna, R., Passamonti, S. and Bernardi, P. (1994) J. Biol. Chem. 269, 16638–16642
40. Scorrano, L., Petronilli, V. and Bernardi, P. (1997) J. Biol. Chem. 272, 12295–12299
41. Soverijn, J.H.M., Huisman, L.A., Rosing, J. and Kemp, N. (1973) Biochim. Biophys. Acta 305, 185–198
42. Klingenberg, M. (1980) J. Membr. Biol. 56, 97–105
43. Krämer, R. and Klingenberg, M. (1982) Biochemistry 21, 1082–1089
44. Costantini, P., Chernyak, B.V., Petronilli, V. and Bernardi, P. (1996) J. Biol. Chem. 271, 6746–6751
45. Chernyak, B.V. and Bernardi, P. (1996) Eur. J. Biochem. 238, 623–630
46. Eriksson, O., Fontaine, E., Petronilli, V. and Bernardi, P. (1997) FEBS Lett. 409, 361–364
47. Eriksson, O., Fontaine, E. and Bernardi, P. (1998) J. Biol. Chem. 273, 12669–12674
48. Woodfield, K., Rück, A., Brdiczka, D. and Halestrap, A.P. (1998) Biochem. J. 336, 287–290
49. Brustovetsky, N. and Klingenberg, M. (1996) Biochemistry 35, 8483–8488
50. Rück, A., Dolder, M., Wallimann, T. and Brdiczka, D. (1998) FEBS Lett. 426, 97–101
51. Halestrap, A.P. (1987) Biochem. J. 244, 159–164
52. Zamzami, N., Brenner, C., Marzo, I., Susin, S.A. and Kroemer, G. (1998) Oncogene 16, 2265–2282
53. Kroemer, G., Dallaporta, B. and RescheRigon, M. (1998) Annu. Rev. Physiol. 60, 619–642
54. Beutner, G., Ruck, A., Riede, B., Welte, W. and Brdiczka, D. (1996) FEBS Lett. 396, 189–195
55. McEnery, M.W., Snowman, A.M., Trifiletti, R.R. and Snyder, S.H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 3170–3174
56. Halestrap, A.P. (1991) Biochem. J. 278, 715–719
57. Bernardi, P., Vassanelli, S., Veronese, P., Colonna, R., Szabo, I. and Zoratti, M. (1992) J. Biol. Chem. 267, 2934–2939
58. Broekemeier, K.M., Schmid, P.C., Schmid, H.H.O. and Pfeiffer, D.R. (1985) J. Biol. Chem. 260, 105–113

59. Broekemeier, K.M. and Pfeiffer, D.R. (1995) Biochemistry 34, 16440–16449
60. Fontaine, E., Eriksson, O., Ichas, F. and Bernardi, P. (1998) J. Biol. Chem. 273, 12662–12668
61. Halestrap, A.P., Griffiths, E.J. and Connern, C.P. (1993) Biochem. Soc. Trans. 21, 353–358
62. Reimer, K.A. and Jennings, R.B. (1992) in The Heart and Cardiovascular System, 2nd edn. (Fozzard, H.A., Jennings, R.B., Huber, E., Katz, A.M. and Morgan, H.E., eds.), pp. 1875–1973, Raven Press, New York
63. Lemasters, J.J. and Thurman, R.G. (1995) Gastroenterology 108, 1317–1320
64. Halestrap, A.P., Connern, C.P., Griffiths, E.J. and Kerr, P.M. (1997) Mol. Cell. Biochem. 174, 167–172
65. Halestrap, A.P., Wang, X., Poole, R.C., Jackson, V.N. and Price, N.T. (1997) Am. J. Cardiol. 80, 17A–25A
66. Lazdunski, M., Frelin, C. and Vigne, P. (1985) J. Mol. Cell. Cardiol. 17, 1029–1042
67. Vandenberg, J.I., Metcalfe, J.C. and Grace, A.A. (1993) Circ. Res. 72, 993–1003
68. Silverman, H.S. and Stern, M.D. (1994) Cardiovasc. Res. 28, 581–597
69. Haigney, M.C.P., Miyata, H., Lakatta, E.G., Stern, M.D. and Silverman, H.S. (1992) Circ. Res. 71, 547–557
70. Griffiths, E.J., Stern, M.D. and Silverman, H.S. (1997) Am. J. Physiol. 273, C37–C44
71. Miyata, H., Lakatta, E.G., Stern, M.D. and Silverman, H.S. (1992) Circ. Res. 71, 605–613
72. Stone, D., Darley-Usmar, V., Smith, D.R. and Oleary, V. (1989) J. Mol. Cell. Cardiol. 21, 963–973
73. Boveris, A., Cadenas, E. and Stoppani, A.O.M. (1976) Biochem. J. 156, 435–444
74. Turrens, J.F., Alexandre, A. and Lehninger, A.L. (1985) Arch. Biochem. Biophys. 237, 408–414
75. Omar, B., McCord, J. and Downey, J. (1991) in Oxidative Stress: Oxidants and Antioxidants (Sies, H., ed.), pp. 493–527, Academic Press, San Diego
76. Nishino, T. (1994) J. Biochem. 116, 1–6
76a. Van Bilsen, M., van der Vusse, G.J, Snoeckx, L.H.E.H., Arts, T., Coumans, W.A, Willemsen, P.H.M. and Reneham, R.S. (1988) Pflügers Arch. 413, 167–173
77. Nazareth, W., Yafei, N. and Crompton, M. (1991) J. Mol. Cell. Cardiol. 23, 1351–1354
78. Qian, T., Nieminen, A.L., Herman, B. and Lemasters, J.J. (1997) Am. J. Physiol. 273, C1783–C1792
79. Broekemeier, K.M., Carpenter-Deyo, L., Reed, D.J. and Pfeiffer, D.R. (1992) FEBS Lett. 304, 192–194
80. Nieminen, A.L., Saylor, A.K., Tesfai, S.A., Herman, B. and Lemasters, J.J. (1995) Biochem. J. 307, 99–106
81. Pastorino, J.G., Snyder, J.W., Hoek, J.B. and Farber, J.L. (1995) Am. J. Physiol. 268, C676–C685
82. Friberg, H., Ferrand-Drake, M., Bengtsson, F., Halestrap, A.P. and Weiloch, T. (1998) J. Neurosci. 18, 5151–5159
83. Griffiths, E.J. and Halestrap, A.P. (1993) J. Mol. Cell. Cardiol. 25, 1461–1469
84. Shimizu, S., Kamiike, W., Hatanaka, N., Miyata, R., Inoue, T., Yoshida, Y., Tagawa, K. and Matsuda, H. (1994) Transplantation 57, 1562–1566
85. Wasaki, S., Sakaida, I., Uchida, K., Kimura, T., Kayano, K. and Okita, K. (1997) Liver 17, 107–114
86. Nieminen, A.L., Byrne, A.M., Herman, B. and Lemasters, J.J. (1997) Am. J. Physiol. 271, C1286–C1294
87. Kerr, P.M., Suleiman, M.-S. and Halestrap, A.P. (1998) Am. J. Physiol. 276, in the press
88. Piper, H.M., Noll, T. and Siegmund, B. (1994) Cardiovasc. Res. 28, 1–15
88. Reimer, M.A., Murry, C.E. and Richard, V.J. (1989) J. Mol. Cell. Cardiol. 21, 1225–1239
89. Lochner, A., van der Merwe, N., de Villiers, M., Steinmann, C. and Kotzyé, J.C. (1987) Biochim. Biophys. Acta 927, 8–17
90. Ferrari, R. (1996) J. Cardiovasc. Pharmacol. 28, S1–S10

91. Opie, L. (1992) Cardiovasc. Res. 2(
92. Massoudy, P., Becker, B.F., Seligm 577–582
93. Peng, C.F., Kane, J.J., Straus, K.D. 45–54
94. Ferrari, R., diLisa, F., Raddino, R.
95. Grover, G.J., Dzwonczyk, S. and S
96. Figueredo, V.M., Dresdner, K.P., V 25, 337–342
97. Benzi, R.H. and Lerch, R. (1992) C
98. Poole-Wilson, P.A. (1989) Mol. Ce
99. Kitakaze, M., Takashima, S., Funay and Hori, M. (1997) Am. J. Physiol.
100. Koop, A. and Piper, H.M. (1992) J.
101. Bond, J.M., Chacon, E., Herman, B C129–C137
102. Atsma, D.E., Bastiaanse, E.M.L., V: Physiol. 270, H2149–H2156
103. Bonventre, J.V. and Cheung, J.Y. (1
104. Gores, G.J., Nieminen, A., Fleishm: (1988) Am. J. Physiol. 255, C315–C
105. Gores, G.J., Nieminen, A., Wray, B 83, 386–396
106. Currin, R.T., Gores, G.J., Thurman
107. Bunger, R., Mallet, R.T. and Hartm
108. Cavallini, L., Valente, M. and Rigob
109. Deboer, L.W.V., Bekx, P.A., Han, I H1571–H1576
110. Borle, A.B. and Stanko, R.T. (1996)
111. Cicalese, L., Lee, K., Schraut, W., W 171, 97–100
112. Desagher, S., Glowinski, J. and Pren
113. Rigobello, M.P., Turcato, F. and Bir
114. Bindoli, A., Callegaro, M.T., Barzor Biochem. Biophys. 342, 22–28
115. Bindoli, A., Barzon, E. and Rigobell
116. Poole, R.C. and Halestrap, A.P. (19
117. Kerr, P.M., Suleiman, M.S. and Hale
118. Cross, H.R., Clarke, K., Opie, L.H. 1369–1381
119. Kerr, P.M., Suleiman, M.S. and Hale
120. Bryson, H.M., Fulton, B.R. and Fau
121. Green, T.R., Bennett, S.R. and Nelso
122. Murphy, P.G., Bennett, J.R., Myers, Anaesthes. 10, 261–266
123. Eriksson, O. (1991) FEBS Lett. 279,
124. Sztark, F., Ichas, F., Ouhabi, R., Dat
125. Kokita, N., and Hara, A. (1996) Ane
126. Ko, S.H., Yu, C.W., Lee, S.K., Chun Anesthes. Analgesia 85, 719–724
127. Kokita, N., Hara, A., Abiko, Y., Ara Anesthes. Analgesia 86, 252–258

iemistry 34, 16440–16449
P. (1998) J. Biol. Chem. 273, 12662–12668
(1993) Biochem. Soc. Trans. 21, 353–358
art and Cardiovascular System, 2nd edn.
A.M. and Morgan, H.E., eds.), pp.

nterology 108, 1317–1320
Kerr, P.M. (1997) Mol. Cell. Biochem.

/.N. and Price, N.T. (1997) Am. J.

fol. Cell. Cardiol. 17, 1029–1042
1993) Circ. Res. 72, 993–1003
c. Res. 28, 581–597
, M.D. and Silverman, H.S. (1992) Circ.

997) Am. J. Physiol. 273, C37–C44
nan, H.S. (1992) Circ. Res. 71, 605–613
ary, V. (1989) J. Mol. Cell. Cardiol. 21,

976) Biochem. J. 156, 435–444
1985) Arch. Biochem. Biophys. 237,

xidative Stress: Oxidants and
ic Press, San Diego

E.H., Arts, T., Coumans, W.A.
igers Arch. 413, 167–173
) J. Mol. Cell. Cardiol. 23, 1351–1354
ters, J.J. (1997) Am. J. Physiol. 273,

J. and Pfeiffer, D.R. (1992) FEBS Lett.

an, B. and Lemasters, J.J. (1995) Biochem.

er, J.L. (1995) Am. J. Physiol. 268,

alestrap, A.P. and Weiloch, T. (1998) J.

Cell. Cardiol. 25, 1461–1469
R., Inoue, T., Yoshida, Y., Tagawa, K. and
6
uyano, K. and Okita, K. (1997) Liver 17,

masters, J.J. (1997) Am. J. Physiol. 271,

1998) Am. J. Physiol. 276, in the press
rdiovasc. Res. 28, 1–15
9) J. Mol. Cell. Cardiol. 21, 1225–1239
teinmann, C. and Kotzyé, J.C. (1987)

1–S10

91. Opie, L. (1992) Cardiovasc. Res. 26, 20–24
92. Massoudy, P., Becker, B.F., Seligmann, C. and Gerlach, E. (1995) Cardiovasc. Res. 29, 577–582
93. Peng, C.F., Kane, J.J., Straus, K.D. and Murphy, M.L. (1980) J. Cardiovasc. Pharmacol. 2, 45–54
94. Ferrari, R., diLisa, F., Raddino, R. and Visioli, O. (1983) J. Mol. Cell. Cardiol. 14, 737–740
95. Grover, G.J., Dzwonczyk, S. and Sleph, P.G. (1990) J. Cardiovasc. Pharmacol. 16, 783–789
96. Figueredo, V.M., Dresdner, K.P., Wolney, A.C. and Keller, A.M. (1991) Cardiovasc. Res. 25, 337–342
97. Benzi, R.H. and Lerch, R. (1992) Circ. Res. 71, 567–576
98. Poole-Wilson, P.A. (1989) Mol. Cell. Biochem. 89, 151–155
99. Kitakaze, M., Takashima, S., Funaya, H., Minamino, T., Node, K., Shinozaki, Y., Mori, H. and Hori, M. (1997) Am. J. Physiol. 272, H2071–H2078
100. Koop, A. and Piper, H.M. (1992) J. Mol. Cell. Cardiol. 24, 55–65
101. Bond, J.M., Chacon, E., Herman, B. and Lemasters, J.J. (1993) Am. J. Physiol. 265, C129–C137
102. Atsma, D.E., Bastiaanse, E.M.L., VanderValk, L. and VanderLaarse, A. (1996) Am. J. Physiol. 270, H2149–H2156
103. Bonventre, J.V. and Cheung, J.Y. (1985) Am. J. Physiol. 249, C149–C159
104. Gores, G.J., Nieminen, A., Fleishman, K.E., Dawson, T.L., Herman, B. and Lemasters, J.J. (1988) Am. J. Physiol. 255, C315–C322
105. Gores, G.J., Nieminen, A., Wray, B.E., Herman, B. and Lemasters, J.J. (1989) J. Clin. Invest. 83, 386–396
106. Currin, R.T., Gores, G.J., Thurman, R.G. and Lemasters, J.J. (1991) FASEB J. 5, 207–210
107. Bunger, R., Mallet, R.T. and Hartman, D.A. (1989) Eur. J. Biochem. 180, 221–233
108. Cavallini, L., Valente, M. and Rigobello, M.P. (1990) J. Mol. Cell. Cardiol. 22, 143–154
109. Deboer, L.W.V., Bekx, P.A., Han, L.H. and Steinke, L. (1993) Am. J. Physiol. 265, H1571–H1576
110. Borle, A.B. and Stanko, R.T. (1996) Am. J. Physiol. 270, G535–G540
111. Cicalese, L., Lee, K., Schraut, W., Watkins, S., Borle, A. and Stanko, R. (1996) Am. J. Surg. 171, 97–100
112. Desagher, S., Glowinski, J. and Premont, J. (1997) J. Neurosci. 17, 9060–9067
113. Rigobello, M.P., Turcato, F. and Bindoli, A. (1995) Arch. Biochem. Biophys. 319, 225–230
114. Bindoli, A., Callegaro, M.T., Barzon, E., Benetti, M. and Rigobello, M.P. (1997) Arch. Biochem. Biophys. 342, 22–28
115. Bindoli, A., Barzon, E. and Rigobello, M.P. (1995) Cardiovasc. Res. 30, 821–824
116. Poole, R.C. and Halestrap, A.P. (1993) Am. J. Physiol. 264, C761–C782
117. Kerr, P.M., Suleiman, M.S. and Halestrap, A.P. (1997) J. Physiol. (London) 501P, P136
118. Cross, H.R., Clarke, K., Opie, L.H. and Radda, G.K. (1995) J. Mol. Cell. Cardiol. 27, 1369–1381
119. Kerr, P.M., Suleiman, M.S. and Halestrap, A.P. (1997) J. Physiol. (London) 499P, P14–P15
120. Bryson, H.M., Fulton, B.R. and Faulds, D. (1995) Drugs 50, 513–519
121. Green, T.R., Bennett, S.R. and Nelson, V.M. (1994) Toxicol. Appl. Pharmacol. 129, 163–169
122. Murphy, P.G., Bennett, J.R., Myers, D.S., Davies, M.J., and Jones, J.G. (1993) Eur. J. Anaesthes. 10, 261–266
123. Eriksson, O. (1991) FEBS Lett. 279, 45–48
124. Sztark, F., Ichas, F., Ouhabi, R., Dabadie, P. and Mazat, J.P. (1995) FEBS Lett. 368, 101–104
125. Kokita, N., and Hara, A. (1996) Anesthesiology 84, 117–127
126. Ko, S.H., Yu, C.W., Lee, S.K., Chung, M.J., Kwak, Y.G., Chae, S.W. and Song, H.S. (1997) Anesthes. Analgesia 85, 719–724
127. Kokita, N., Hara, A., Abiko, Y., Arakawa, J., Hashizume, H. and Namiki, A. (1998) Anesthes. Analgesia 86, 252–258

128. Buljubasic, N., Marijic, J., Berczi, V., Supan, D.F., Kampine, J.P. and Bosnjak, Z.J. (1996) Anesthesiology 85, 1092–1099
129. Li, Y.C., Ridefelt, P., Wikiund, L. and Bjerneroth, G. (1997) Acta Anaesthesiol. Scand. 41, 633–638
130. Millar, C.G.M., Baxter, G.F. and Thiemermann, C. (1996) Pharmacol. Therapeut. 69, 143–151
131. Schwarz, E.R., Whyte, W.S. and Kloner, R.A. (1997) Curr. Opin. Cardiol. 12, 475–481
132. Ytrehus, K., Liu, Y.G. and Downey, J.M. (1994) Am. J. Physiol. 266, H1145–H1152
133. Schultz, J.J., Hsu, A.K. and Gross, G.J. (1997) J. Mol. Cell. Cardiol. 29, 1355–1362
134. Meldrum, D.R., Cleveland, J.C., Mitcheli, M.B., Sheridan, B.C., Gamboni Robertson, F., Harken, A.H. and Banerjee, A. (1996) Am. J. Physiol. 271, R718–R726
135. Liang, B.T. (1996) Am. J. Physiol. 271, H1769–H1777
136. Cleveland, J.C., Meldrum, D.R., Cain, B.S., Banerjee, A. and Harken, A.H. (1997) Circulation 96, 29–32
137. Schultz, J.E.J., Yao, Z.H., Cavero, I. and Gross, G.J. (1997) Am. J. Physiol. 272, H2607–H2615
138. Yabe, K., Nasa, Y., Sato, M., Iijima, R. and Takeo, S. (1997) Cardiovasc. Res. 33, 677–685
139. Vuorinen, K., Ylitalo, K., Peuhkurinen, K., Raatikainen, P., Alarami, A. and Hassinen, I.E. (1995) Circulation 91, 2810–2818
140. Vanderheide, R.S., Hill, M.L., Reimer, K.A. and Jennings, R.B. (1996) J. Mol. Cell. Cardiol. 28, 103–112
141. Gottlieb, R.A., Burleson, K.O., Kloner, R.A., Babior, B.M. and Engler, R.L. (1994) J. Clin. Invest. 94, 1621–1628
142. Fliss, H. and Gattinger, D. (1996) Circ. Res. 79, 949–956
143. Bromme, H.J. and Holtz, J. (1996) Mol. Cell. Biochem. 164, 261–275
144. Umansky, S.R. and Tomei, L.D. (1997) Adv. Pharmacol. 41, 383–407
145. Olivetti, G., Quaini, F., Sala, R., Lagrasta, C., Corradi, D., Bonacina, E., Gambert, S.R., Cigola, E. and Anversa, P. (1996) J. Mol. Cell. Cardiol. 28, 2005–2016
146. Olivetti, G., Abbi, R., Quaini, F., Kajstura, J., Cheng, W., Nitahara, J.A., Quaini, E., DiLoreto, C., Beltrami, C.A., Krajewski, S. et al. (1997) N. Engl. J. Med. 336, 1131–1141
147. Long, X.L., Boluyt, M.O., Hipolito, M.D., Lundberg, M.S., Zheng, J.S., O'Neill, L., Cirielli, C., Lakatta, E.G. and Crow, M.T. (1997) J. Clin. Invest. 99, 2635–2643
148. Mignotte, B. and Vayssiere, J.-L. (1998) Eur. J. Biochem. 252, 1–15
149. Susin, S.A., Zamzami, N., Castedo, M., Daugas, E., Wang, H.G., Geley, S., Fassy, F., Reed, J.C. and Kroemer, G. (1997) J. Exp. Med. 186, 25–37
150. Kroemer, G. and Green, D. (1998) Trends Cell Biol. 8, 267–271
151. Zamzami, N., Marchetti, P., Castedo, M., Decaudin, D., Macho, A., Hirsch, T., Susin, S.A., Petit, P.X., Mignotte, B. and Kroemer, G. (1995) J. Exp. Med. 182, 367–377
152. Zamzami, N., Marchetti, P., Castedo, M., Hirsch, T., Susin, S.A., Mignotte, B. and Kroemer, G. (1996) FEBS Lett. 384, 53–57
153. Kluck, R.M., Martin, S.J., Hoffman, B.M., Zhou, J.S., Green, D.R. and Newmeyer, D.D. (1997) EMBO J. 16, 4639–4649
154. Liu, X., Kim, C.N., Yang, J., Jemmerson, R. and Wang, X. (1996) Cell 86, 147–157
155. Kluck, R.M., BossyWetzel, E., Green, D.R. and Newmeyer, D.D. (1997) Science 275, 1132–1136
156. Halestrap, A.P. (1982) Biochem. J. 204, 37–47
157. Yang, J., Liu, X.S., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J.Y., Peng, T.I., Jones, D.P. and Wang, X.D. (1997) Science 275, 1129–1132
158. Kantrow, S.P. and Piantadosi, C.A. (1997) Biochem. Biophys. Res. Commun. 232, 669–671
159. Susin, S.A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M. and Kroemer, G. (1996) J. Exp. Med. 184, 1331–1341

160. Gottschalk, A.R., Boise, L.H., Thom
Sci. U.S.A. 91, 7350–7354
161. Garland, J.M. and Halestrap, A. (199
162. Salvioli, S., Ardizzoni, A., Francesch
163. Garland, J.M., Sondergaard, K.L. an
164. Bossy-Wetzel, E., Newmeyer, D.D.
165. Kitagaki, K., Niwa, S., Hoshiko, K.,
Biophys. Res. Commun. 222, 71–77
166. McDonald, J.W., Goldberg, M.P., G
40, 756–758
167. Mosieniak, G., Figiel, I. and Kaminsk
168. Leist, M. and Nicotera, P. (1997) Bio
169. Leist, M., Single, B., Castoldi, A.F., K
1481–1486

Kampine, J.P. and Bosnjak, Z.J. (1996)

G. (1997) Acta Anaesthesiol. Scand. 41,

. (1996) Pharmacol. Therapeut. 69,

7) Curr. Opin. Cardiol. 12, 475–481
m. J. Physiol. 266, H1145–H1152
ol. Cell. Cardiol. 29, 1355–1362
ieridan, B.C., Gamboni Robertson, F.,
ol. 271, R718–R726
777
ee, A. and Harken, A.H. (1997)

J. (1997) Am. J. Physiol. 272,

5. (1997) Cardiovasc. Res. 33, 677–685
inen, P., Alarami, A. and Hassinen, I.E.

nnings, R.B. (1996) J. Mol. Cell. Cardiol.

or, B.M. and Engler, R.L. (1994) J. Clin.

)–956
iem. 164, 261–275
nacol. 41, 383–407
adi, D., Bonacina, E., Gambert, S.R.,
iol. 28, 2005–2016
ng, W., Nitahara, J.A., Quaini, E.,
997) N. Engl. J. Med. 336, 1131–1141
erg, M.S., Zheng, J.S., O'Neill, L.,
Clin. Invest. 99, 2635–2643
chem. 252, 1–15
, Wang, H.G., Geley, S., Fassy, F., Reed,
)7
l. 8, 267–271
n, D., Macho, A., Hirsch, T., Susin, S.A.,
Exp. Med. 182, 367–377
r., Susin, S.A., Mignotte, B. and

S., Green, D.R. and Newmeyer, D.D.

ang, X. (1996) Cell 86, 147–157
ewmeyer, D.D. (1997) Science 275,

A.M., Cai, J.Y., Peng, T.I., Jones, D.P. and

n. Biophys. Res. Commun. 232, 669–671
Marchetti, P., Macho, A., Daugas, E.,
. 184, 1331–1341

160. Gottschalk, A.R., Boise, L.H., Thompson, C.B. and Quintans, J. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7350–7354
161. Garland, J.M. and Halestrap, A. (1997) J. Biol. Chem. 272, 4680–4688
162. Salvioli, S., Ardizzoni, A., Franceschi, C. and Cossarizza, A. (1997) FEBS Lett. 411, 77–82
163. Garland, J.M., Sondergaard, K.L. and Jolly, J. (1997) Br. J. Haematol. 99, 756–765
164. Bossy-Wetzel, E., Newmeyer, D.D. and Green, D.R. (1998) EMBO J. 17, 37–49
165. Kitagaki, K., Niwa, S., Hoshiko, K., Nagai, H., Hayashi, S. and Totsuka, T. (1996) Biochem. Biophys. Res. Commun. 222, 71–77
166. McDonald, J.W., Goldberg, M.P., Gwag, B.J., Chi, S.I. and Choi, D.W. (1996) Ann. Neurol. 40, 750–758
167. Mosieniak, G., Figiel, I. and Kaminska, B. (1997) J. Neurochem. 68, 1142–1149
168. Leist, M. and Nicotera, P. (1997) Biochem. Biophys. Res. Commun. 236, 1–9
169. Leist, M., Single, B., Castoldi, A.F., Kuhnle, S. and Nicotera, P. (1997) J. Exp. Med. 185, 1481–1486